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Updated Mutagenicity and Carcinogenicity Assessment of Cadmium: Addendum to the Health Assessment Document for Cadmium (May 1981) EPA-600/8-81-023

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16. ABSTRACT

This draft document evaluates the mutagenicity and carcinogenicity of cadmium, supplementing an earlier document (Health Assessment Document for Cadmium, May 1981) which dealt with all health effects. Since the earlier document was prepared, a rat inhalation carcinogenicity study has been reported and several epidemiology and mutagenicity papers have been published.

This draft document tentatively concludes that: (1) there is evidence suggesting that cadmium and certain cadmium compounds are weakly mutagenic; (2) cadmium chloride aerosol induces lung cancer in rats; (3) injected cadmium salts induce injection site sarcomas and testicular tumors in both mice and rats; (4) there is limited epidemiologic evidence that inhaled cadmium induces prostate and/or lung cancer in highly exposed workers; (5) there is no evidence that cadmium is carcinogenic via ingestion, which is a major route of human exposure, and the upper-limit of potency via ingestion is at least 200 times less than via inhalation.

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SUMMARY AND CONCLUSIONS

SUMMARY

Qualitative Assessment

As was noted in the Office of Health and Environmental Assessment Health Assessment Document for Cadmium (May 1981), cadmium and various cadmium salts have produced injection site sarcomas in rats and interstitial cell testicular tumors in mice and rats after subcutaneous injection. Specifically, injections site sarcomas were produced by cadmium powder, cadmium sulfide, cadmium oxide, cadmium sulfate, and cadmium chloride, and interstitial cell tumors were produced by cadmium chloride, cadmium sulfate, and ferritin containing cadmium. However, three drinking water, two gavage, and two dietary studies using cadmium acetate, cadmium sulfate, or cadmium chloride have shown no excessive risk of cadmium carcinogenicity in rats and mice. It was also noted that human exposure to high levels of cadmium dust and/or fumes in smelter and battery plants produced a slight, but statistically significant, increase in prostate cancer in several different small-scale epidemiologic studies.

After the above document was prepared, a lifetime rat inhalation study was published showing a dose-related induction of lung carcinomas by cadmium chloride aerosol. The carcinogenic potency of cadmium by chronic inhalation in the rat appears to be at least two orders of magnitude greater than by chronic oral administration. In another recent study, lifetime observation of rats given cadmium oxide by intratracheal instillation did not reveal a carcinogenic effect; however, only three or fewer treatments were given during the study, which involved a relatively low dose.

Seven epidemiologic studies reviewed after the May 1981 OHEA document have not appreciably changed the earlier limited evidence of prostate cancer in humans. In addition to cancer of the prostate, the risk of lung cancer was found to be significantly correlated with cadmium exposure in two studies (Lemen et al. 1976, Holden 1980). The author of one attributes the excess risk to the presence of arsenic (Holden 1980), although arsenic was present in both studies. Because of the presence of arsenic along with cadmium in these studies, the evidence that cadmium is a causitive agent of lung cancer is inadequate.

A clear statement on the mutagenicity of cadmium is rather difficult because of the conflicting results and lack of adequate test protocols used. However, gene mutation studies in mammalian cell cultures, rec-assays in bacteria, chromosomal nondisjunction studies in intact mammals, and other indirect evidence reported in the text suggest that cadmium is weakly mutagenic.

Quantitative Assessment

Since humans are exposed to cadmium dust or fumes and rats were exposed to cadmium chloride aerosol, a limitation inherent in the use of the rat study in estimating human risk is the possible difference between humans and rats in the lung retention of particulates or between the biological effectiveness of cadmium chloride aerosol administered to rats and the dust and fumes inhaled by workers. Since data are not clear on this point, assumptions of equal lung uptake and equal effectiveness were made in order to get an idea of the human risk.

Given these assumptions, combined with other assumptions and conventions used in quantitative risk assessment procedures, the Takenaka et al. (1982) data on lung carcinomas in rats during lifetime inhalation exposures to cadmium chloride aerosol were analyzed. The result of the analysis is that the upper-bound cancer risk to humans who continuously breathe 1 ug/m^3 of elemental cadmium for a lifetime is 0.15.

Based on respiratory and prostate cancer rates from the Lemen et al. (1976) study of cadmium smelter workers, the upper-bound cancer risk from lifetime exposure to 1 ug/m^3 of cadmium in the air has a range of 1.9×10^{-3} to 2.5×10^{-3}. These estimates are about 100 times less than the estimate of lung cancer risks from the rat experiment. Because only fragmentary information is available concerning cadmium exposures of the workers, the extreme range of risk, based on the highest and lowest possible assumptions about several exposure parameters, is a factor of 100 higher and lower than these estimates. Further detailed analysis and laboratory studies are needed before the large difference between the estimates based on animal and human data are resolved.

CONCLUSIONS

Applying the International Agency for Research on Cancer (IARC) approach (Appendix B) for classifying the weight-of-evidence for carcinogenicity in experimental animals, the injection site and testicular tumors in mice and rats given cadmium metal or cadmium salts and the lung carcinomas in rats exposed to cadmium chloride aerosol by inhalation provide sufficient evidence for the carcinogenicity of cadmium and certain cadmium compounds in experimental animals. Although there is a possibility that orally-ingested cadmium is carcinogenic in rats based on the finding of testicular tumors induced by injection at a remote site, no response has been observed with ingested cadmium, and the potency via the oral route is at least 200 times less than via inhalation.

The available human epidemiologic data provide limited evidence, according to the IARC criteria, that cadmium and certain cadmium compounds are carcinogenic in humans.

The overall evidence for carcinogenicity, applying the IARC criteria, places cadmium and certain cadmium compounds in the 2A category, meaning that they are probably carcinogenic in humans.

The upper-bound unit risk estimate for continuous inhalation exposure at a cadmium concentration of 1 ug/m^3 ranges from 1.9×10^{-3} to 2.5×10^{-3} based on lung and prostate cancer, respectively, from one smelter worker study, although there is considerable uncertainty in these estimates due to uncertain exposure of the workers. Nevertheless, these estimates are regarded as more realistic than the estimate based on the rat inhalation study, which is about 100 times higher.

INTRODUCTION

This document is a review and assessment of the current information relating to the mutagenicity and carcinogenicity of cadmium. It contains a detailed discussion of information on those subjects which became available since the earlier Health Assessment Document for Cadmium was prepared by the Office of Health and Environmental Assessment (OHEA) in May 1981. It includes all pertinent material from the 1981 document but does not attempt to repeat details of the animal carcinogenicity studies discussed there.

MUTAGENICITY

Cadmium has been investigated for its mutagenic potential in both prokaryotic and eukaryotic systems. The prokaryotic systems include assays for gene mutation and reparable genetic damage in bacteria. The eukaryotic systems include gene mutation studies in yeast, *Drosophila*, and mammalian cells and chromosomal aberration studies in human and other mammalian cells exposed to cadmium both *in vitro* and *in vivo*. The following is an analysis of the literature pertaining to the mutagenic effects of cadmium.

GENE MUTATIONS IN PROKARYOTES

Gene mutation studies in prokaryotic systems have been summarized in Table 1. A discussion of each study follows.

Salmonella Assay

Heddle and Bruce (1977) and Bruce and Heddle (1979) tested the mutagenic effects of cadmium chloride in the histidine reverse mutation assay using *Salmonella typhimurium* tester strains TA100, TA98, and TA1537. The test compound (purchased from ICN Pharmaceuticals, Plainview, New York) was dissolved in water and used at concentrations of 0.05, 0.5, 5, 50, and 500 ug/plate with and without the application of a metabolic activation system (S9 mix) derived from phenobarbital-induced rat liver homogenate. According to these authors cadmium chloride did not induce a significant mutagenic response over the control value. The criterion set for a positive response was 50% or 1.5-fold increase in the revertant frequency over the negative control or spontaneous frequency. Revertant counts were given only for strain TA100; the spontaneous frequency of revertants in this strain was 140 colonies per plate. The purity of cadmium chloride was not given in this report.

TABLE 1. MUTAGENICITY EVALUATION OF CADMIUM: GENE MUTATIONS IN PROKARYOTES

Test System	Strain	Cadmium Compound	Dose	S9 Activation System	Results	Comments	Reference
Salmonella typhimurium	TA98 TA100 TA1535 TA1537 TA1538	Cadmium chloride aqueous solution	0.05 0.5 5.0 50.0 500 ug/plate	Phenobarbital-induced rat liver	Reported as negative	1. Data are not presented clearly as revertants/plate for each strain. 2. Purity of compound not discussed.	Bruce and Heddle (1979)
Salmonella typhimurium	TA98 TA1535 TA1537	Cadmium red in DMSO	1 ug/ml	Aroclor 1254-induced mouse liver	Reported as negative	1. Data provided only for the preincubation or suspension assay. No data on the spot test given. 2. Only a single dose was employed; no dose-response data.	Milvy and Kay (1978)
Salmonella typhimurium	TA1535 TA1537	Cadmium chloride (solvent not specified)	10, 20, 30, 45, 90 mM	Uninduced mouse liver	Reported as negative	1. Spontaneous reversion data and the experimental reversion data have not been given in terms of numbers. 2. Used uninduced mouse liver S9 activation system. 3. No positive controls.	Polukhina et al. (1977)

(continued on the following page)

TABLE 1. (continued)

Test System	Strain	Cadmium Compound	Dose	S9 Activation System	Results	Comments	Reference
Salmonella typhimurium	TA98 TA100 TA1535 TA1537 TA1538	Cadmium diethylthiocarbamate in DMSO	1 5 10 50 100 ug/plate	Aroclor-induced rat liver	Reported positive for TA1538 and TA98 in the absence of S9 activation. Reported weakly positive both in the presence and absence of S9 activation.	1. Lowest effective dose was 10 ug/plate. 2. Reported positive only for one dose. 3. No dose-response relatonship.	Hedenstedt et al. (1979)
Bacillus subtilis Rec-assay	H17 Rec⁺ M45 Rec⁻	Cadmium chloride aqueous solution	0.05 M/plate	None	Reported as weakly (+) positive		Nishioka (1975)
		Cadmium nitrate aqueous solution			Reported as negative		

(continued on the following page)

TABLE 1. (continued)

Test System	Strain	Cadmium Compound	Dose	S9 Activation System	Results	Comments	Reference
Bacillus subtilis Rec-assay	H17 Rec$^+$ M45 Rec$^-$	Aqueous solutions of cadmium chloride, nitrite, and sulfite	0.005 M/plate	None	Reported as weakly (+) positive	1. Compounds were pure.	Kanematsu et al. (1980)

In an abstract published by Kalinina and Polukhina (1977), cadmium chloride was reported to be nonmutagenic in the Salmonella assay. However, important variables such as the number of strains used, the dosage employed, and the number of revertants per plate were not reported. Polukhina et al. (1977) also reported negative results with cadmium chloride on Salmonella typhimurium strains TA1535 and TA1537 both in the presence and absence of an S9 activation system derived from uninduced mouse liver homogenate. In this report a suspension assay with cadmium chloride concentrations of 10, 20, 30, 45, and 90 mM was employed. Positive and negative control data were not presented in this paper, so it is not possible to know whether or not the assay system was functioning properly. Toxicity of the test compound was not reported by these investigators.

Milvy and Kay (1978) studied the mutagenic effects of cadmium red (cadmium sulfide and selenium), a dye used in the printing industry, using the Salmonella spot test (Ames et al. 1973) and the preincubation assay (Ames et al. 1975). Salmonella typhimurium strains TA1538, TA98, and TA1535 were employed in these studies. The test compound (10 ug) was dissolved in 0.01 ml dimethyl sulfoxide (DMSO) and added to 0.9 ml of incubation mixture for 30 minutes at 37°C with shaking before plating 0.1 ml onto minimal plates. Experiments were carried out both in the presence and absence of an S9 activation system derived from Aroclor 1254-induced mouse liver homogenate. Cadmium red was reported to be nonmutagenic in both tests. However, data are presented only for the suspension assay. These investigators used only one concentration, and hence, no dose-response relationship was demonstrated. The toxicity of the compound for each strain was not reported. Consequently, this study may be regarded as inconclusive.

Hedenstedt et al. (1979) studied the mutagenic effects of cadmium diethyldithiocarbamate (used in rubber and plastic industries) in Salmonella typhiumurium strains TA1535, TA1537, TA1538, TA98, and TA100. The concentrations used were 1, 5, 10, 50, and 100 ug/plate. The compound was dissolved in DMSO. Concentrations of 50 and 100 ug/plate were toxic in many of these strains. The concentration of 10 ug/plate exhibited mutagenic activity in strains TA1538 and TA98 in the absence of a metabolic activation (S9) system obtained from Aroclor 1254-induced rat liver homogenate (Ames et al. 1975). In TA 1538 the revertant frequency increased more than twofold at 10 ug/plate, i.e., 26.3 ± 3.7 revertants/plate compared to a control value of 11.8 ± 2.6 revertants/plate in the absence of metabolic activation. In the presence of metabolic activation the revertant frequencies in treated and controls were the same. In TA98, the revertant frequency was 58.8 ± 2.3 at 10 ug/plate (almost a twofold increase) compared to the control frequency of 31.5 ± 4.2 revertants/plate in the absence of metabolic activation. No data were given for studies in the presence of metabolic activation. Positive control data were not presented even though the authors indicate that positive controls were employed in the experiment. These results may be regarded as an inconclusive because the twofold increase was observed for only one isolated data point, and there was no evidence of dose-response.

Escherichia coli WP2 Assay

Venitt and Levy (1974), in a report on the mutagenicity of chromates in the Escherichia coli WP2 forward mutation system, mentioned that they also tested cadmium compounds for mutagenicity and found them to be negative. These authors do not mention what types of cadmium compounds they employed or present data to support their negative conclusions.

Bacillus subtilis Rec-Assay

Nishioka (1975) investigated the mutagenicity of cadmium chloride and cadmium nitrate using the rec-assay of Kada et al. (1972). In the rec-assay, which measures reparable DNA damage, differences in growth sensitivities of Bacillus subtilis strains H17 (recombination-competent wild type rec$^+$) and M45 (recombination-deficient rec$^-$) to mutagenic chemicals are measured. When a chemical is more inhibitory to rec$^-$ than to rec$^+$ cells, then it is suspected of being mutagenic. Concentrations of 2.5×10^{-7} cells/0.1 ml were streaked on agar plates from the center of the plate in different directions. Aqueous solutions of cadmium chloride and cadmium nitrate solutions (0.05 M) were applied in 0.05 ml aliquots to filter paper disks (diameter of 10 mm) and placed in the center of the agar plate, i.e., the starting point of the streaks of rec$^+$ and rec$^-$ cells. All plates were incubated at 37°C for 24 hours. The inhibition of growth was indicated by the distance (mm) between the paper disk and growth of the bacterial streaks. This magnitude of inhibition is called "rec-effect" and is expressed as: no difference between rec$^+$ and rec$^-$ plates (-), less than 5 mm difference (+), 5-10 mm difference (++), and more than 10 mm difference (+++). Cadmium nitrate showed no difference in growth inhibition (-), whereas cadmium chloride exhibited a weak positive response (+). No positive or negative controls were employed in this experiment. Each experiment was repeated three times. These experiments did not use a metabolic activation system. The cadmium compounds used were of reagent grade but their precise purity was not reported.

Similar results were obtained by Kanematsu et al. (1980) in the rec-assay of Kada et al. (1972). Cadmium chloride, cadmium nitrite, and cadmium sulfate were employed at a concentration of 0.005 M in 0.05 ml aqueous solution. All these compounds exhibited a weak mutagenic response (+) (of 4-5 mm growth inhibition zone). According to these authors, test compounds used were of the highest purity commercially available in Japan.

GENE MUTATIONS IN YEAST

Cadmium chloride has been investigated for the induction of gene mutations in the yeast Saccharomyces cerevisiae (Table 2) (Takahashi 1972; Putrament et al. 1977). Takahashi (1972) studied the induction of petite mutations (p-mutation) and auxotrophs in the Saccharomyces cerevisiae heterozygous diploid strain C3116. He treated 10^4 cells with 10 (5.5×10^{-5}M), 12 (6.6×10^{-5}M), and 20 ppm (1.1×10^{-4}M) for 2 days (48 hours) at 25°C. After 2 days of growth, the cell number was determined and the cell suspension was diluted to give a concentration of 2.8×10^3 cells per ml. One-tenth of the diluted suspension was spread on the YEPD-agar plate and incubated at 28°C. When small colonies appeared on the plate, they were replica plated onto YEP-glycerol-agar medium and minimal medium. After overnight incubation at 28°C, induced p-mutants and auxotrophs were scored. At the dose of 12 ppm (1.1×10^{-4}M), no p-mutants or auxotrophs were found in the 786 colonies counted; at the dose of 10 ppm, 10 p-mutants and three auxotrophs were detected in the 871 colonies counted; and at the dose of 20 ppm, there were 12 p-mutants and 9 auxotrophs in 1,182 colonies indicating that cadmium chloride may be mutagenic. In the controls there were five p-mutants and two auxotrophs in 2,875 colonies counted. According to this paper, however, mutants were induced at the dosage of 10 ppm and 20 ppm but not at the dosage of 12 ppm. Such erratic fluctuations in mutation frequency and the low number of mutants suggest that the positive values may similarly be questionable. Since p-mutants occur by damage involving the mitochondrial DNA rather than

TABLE 2. MUTAGENICITY EVALUATION OF CADMIUM: GENE MUTATIONS IN YEAST AND MAMMALIAN CELL CULTURES

Test System	Strain	Cadmium Compound	Dose	Activation System	Results	Comments	Reference
Saccharomyces cerevisiae (Yeast) P-mutants and auxotrophs	C3116	Cadmium chloride	10 12 20 ppm	None	Reported as positive	1. P-mutants may not represent true gene mutations because they arise by damage in mitochondrial DNA. 2. Vague protocol.	Takahashi (1972)
Saccharomyces cerevisiae P-mutants	197/2d	Cadmium chloride	8 ppm	None	Reported as negative	1. Only one concentration of test compound was used. 2. This concentrations was too toxic for the cells. 3. No mutants observed in those few suvivors.	Putrament et al. (1977)
Mouse lymphoma	L5178Y TK$^{+/-}$	Cadmium chloride	0.05 0.06 0.08 0.11 0.15 ug/ml	None	Reported as weakly positive	1. Application of t-test to determine the significance has been challenged by Clive et al. (1981).	Amacher and Paillet (1980)
Chinese hamster cells	Lung (Don) cells; resistance to 8-azoguanine	Cadmium acetate Cadmium chloride	2.5 5 10 ug/ml	None	Reported as positive	1. Very low survival due to high toxicity. 2. Observations not repeated and confirmed.	Casto (1976)
Chinese hamster cells	Ovary cells (CHO)	Cadmium chloride	2.5 5 5 10 ug/ml	None	Reported as weakly positive	1. Data not presented.	Hsie et al. (1978)

nuclear DNA, caution should be exercised in assessing the mutagenic potential of chemicals with this system.

Putrament et al. (1977) also reported a negative result in a test for induction of p-mutation by cadmium chloride in Saccharomyces cerevisiae. The concentration of cadmium chloride tested (8 mM) was very toxic, however, and less than 1% of the cells survived a 6-hour incubation in YEP-glucose medium. No increase of p-mutants was observed and no data were presented. This study is regarded as inconclusive.

GENE MUTATIONS IN MAMMALIAN CELL CULTURES

Gene mutation studies in cultured mammalian cells have also been summarized in Table 2. A discussion of each study follows.

Mouse Lymphoma Assay

Amacher and Paillet (1980) reported that cadmium choloride (ICN Phamaceuticals) was mutagenic in the mouse lymphoma L5178Y $TK^{+/-}$ assay. When cadmium chloride, dissolved in normal saline, was tested at concentrations of 2.35×10^{-7}M (cell survival 100 ± 11%), 3.57×10^{-7}M (cell survival 78 ± 24%), 4.5×10^{-7}M (cell survival 0.62 ± 4%), 6.00×10^{-7}M (cell survival 38 ± 11%), and 8.00×10^{-7}M (cell survival 12 ± 1%), there was a dose-related increase in the mutation frequency. The mutation frequencies per 10^4 survivors for the above doses were 0.48 ± 0.01, 0.58 ± 0.06, 0.56 ± 0.05, 0.63 ± 0.16, and 0.68 ± 0.04, respectively. The mutation frequency at the highest nontoxic dosage of 6.00×10^{-7}M was approximately 1.5-fold higher than the control frequency of 0.40 ± 0.03 (survival 100% ± 5). The dose-response curve obtained by Amacher and Paillet has been criticized by Clive et al. (1981), who claim that the application of a t-test for low numbers of samples to determine significance is misleading.

Chinese Hamster Cell Assay

Casto (1976), in a report submitted to Dr. Richard Troast of the Office of Pesticide Programs, U.S. Environmental Proctetion Agency, stated that cadmium acetate and cadmium chloride are mutagenic in Chinese hamster-lung cells (Don) as determined by induction of mutations that confer resistance to 8-azoguanine. Cells were treated with 2.5 (1.36×10^{-8}M), 5 (2.72×10^{-8}M), and 10 ug/ml (5.45×10^{-8}M) of cadmium acetate and cadmium chloride, respectively, for 18 hours followed by 48 hours expression time. Cadmium acetate induced mutation frequencies of 2.8, 50, and 10 per 10^6 survivors, respectively, for above dosages. The survival rate was 0.70, 0.92, and 0.43 percent, respectively. Cadmium chloride induced mutation frequencies of 6, 7, 14, and 37 per 10^6 survivors. The negative control rate was 2 per 10^6 survivors. According to this investigation both cadmium acetate and cadmium chloride are weakly mutagenic, but the results are questionable because of low survival rates at these high concentrations. Hsie et al. (1978) also reported cadmium chloride to be weakly mutagenic at the HGPRT locus in the Chinese hamster ovary cells, but no data were presented. Therefore, although three independent studies have reported cadmium salts to be weakly mutagenic in cultured mammalian cells, however, in none of the studies was the demonstration of mutagenicity unequivocal.

STUDIES IN DROSOPHILA AND OTHER INSECTS

Genetic effects of cadmium in Drosophila are summarized in Table 3. A discussion of each study follows.

TABLE 3. MUTAGENICITY EVALUATION OF CADMIUM: GENE MUTATIONS AND CHROMOSOMAL ABERRATIONS IN DROSOPHILA AND OTHER INSECTS

Test System	Cadmium Compound	Dosage	Treatment Period	Results	Comments	Reference
Drosophila melanogaster sex-linked recessive lethal test	Cadmium chloride	50.0 mg/l (2.72×10^{-4}M)	Larvae feeding	Reported as negative	1. Data not presented. 2. Only one dose was used.	Sorsa and Pfeifer (1973)
Drosophila melanogaster larval development sex chromosome loss sex-linked recessive lethal test	Cadmium chloride	65 mg/l 62 mg/l + 3,000 R x-rays	Larvae feeding	Reported as negative	1. Treatment was done in larvae only.	Ramel and Friberg (1974)
Drosophila melanogaster dominant lethal mutations	Cadmium chloride	5 10 20 ppm	Larvae feeding	Reported as positive	1. Dose-response reported. 2. Confirmation of these results in an independent laboratory would be of interest for comparative purposes.	Vasudev and Krishnamurthy (1979)
Drosophila melanogaster sex-linked recessive lethal test	Cadmium stearate	10-20 mg/l 50-100 mg/l 100 mg/l 3 mg/m^3	5-10 days (feeding larvae) 10-12 days (feeding adults) (feeding larvae) (inhalation adult)	Reported as negative	1. Rationale for selecting the dosage not given.	Sabalina (1968)

(continued on the following page)

TABLE 3. (continued)

Test System	Cadmium Compound	Dosage	Treatment Period	Results	Comments	Reference
Drosophila melanogaster chromosomal nondisjunction sex chromosome loss	Cadmium chloride	62 ppm		Reported as negative	1. No data have been presented.	Ramel and Magnusson (1979)
Drosophila melanogaster sex-linked recessive lethal test	Cadmium chloride aqueous solution	50 ppm	Larvae feeding	Reported as negative	1. Toxicity was determined. 2. Development and survival was affected by cadmium.	Inoue and Watanabe (1978)
Poekilocerus pictus (grasshopper) testis (meiotic chromosomal)	Cadmium chloride aqueous solution	0.001% 0.01% 0.05% per animal		Reported as positive	1. The effect may be cytotoxic rather than genetic. 2. No controls.	Kumaraswamy and Rajasekarasetty (1977)

Sorsa and Pfeifer (1973) reported that cadmium chloride at concentrations of 1.25 (6.81×10^{-6}M), 2.5 (1.36×10^{-5}M), 5.0 (2.72×10^{-5}M), 10.0 (5.45×10^{-5}M), 20.0 (1.09×10^{-4}M), and 50 mg/l (2.27×10^{-4}M) of media caused significant delay in the development of larvae compared to controls. In the sex-linked recessive lethal mutation test (Muller-5 test), only one concentration of 50 mg/l (2.72×10^{-4}M) was used with no indication of mutagenic response. The number of chromosomes tested and the criteria set for scoring the lethals were not reported, however, and no data were presented.

Ramel and Friberg (1974), using a dose of 62 mg (3.32×10^{-4}M) of cadmium chloride/l of media, which was the maximum nonlethal dose in the toxicity test, found a delay in larval development. They also studied the induction of sex chromosome loss and sex-linked recessive lethal mutations. In the sex chromosome loss test, a total of 23,360 chromosomes from the treated group and 28,143 chromosomes from the control group were tested. The frequencies of sex chromosome losses were 0.3% and 0.2% for the treated and the control groups, respectively. In the sex-linked recessive lethal test, a total of 1,045 chromosomes from the treated group and 742 chromosomes from the control group were tested. The frequencies of sex-linked recessive lethals were 8.1% and 6.1% for the treated and control groups, respectively. Although these experiments did not indicate a significant effect of cadmium chloride in the sex chromosome loss or sex-linked recessive lethal tests, the number of chromosomes tested in the sex-linked recessive lethal test was too low to preclude all but a strong mutagenic response.

The mutagenic activity of cadmium sterate was studied by Yu. A. Revazova quoted by Sabalina (1968) in _Drosophila melanogaster_ using the sex-linked recessive lethal test. Flies were fed a medium containing 10-20 mg (5.45×10^{-5}M to 1.09×10^{-4}M) and 50-100 mg (2.72×10^{-4} to

5.45×10^{-4}M) of cadmium stearate/l substrate for 5-10 and 10-12 days, respectively. The number of sex-linked recessive lethal mutations in 805 chromosomes analyzed was 1 (0.12%) for the 5-10 day treatment, and the number of sex-linked recessive lethal mutations in 2,192 chromosomes examined was 8 (0.36%) for the 10-12 day treatment. When larvae were treated with cadmium sterate concentration of 100 mg/l substrate for 12 days and scored for sex-linked recessive lethal mutants in 380 chromosomes, no mutants were discovered. Cadmium sterate was also administered by inhalation to adult flies for 32 hours (4 hours daily for 8 days). The mean cadmium concentration was 3 mg/m^3. The percent sex-linked recessive lethal mutations among the 498 chromosomes was reported to be 0.2%. The control frequency of sex-linked recessive lethal mutations was not provided in the paper. The number of chromosomes tested was not adequate in this study. This study provides no evidence of mutagenicity of cadmium in Drosophila, but the scale of the study was too small to be considered an adequate test even if appropriate controls were presented.

Induction of dominant lethal mutations in Drosophila melanogaster with cadmium chloride has been reported by Vasudev and Krishnamurthy (1979). The doses used were 5 (2.72×10^{-5}M), 10 (5.5×10^{-5}M), and 20 ppm (1.1×10^{-4}M). The frequencies of dominant lethals were 11.8, 14.3, and 14.3%, respectively, in 1,244, 1,375, and 1,390 eggs counted. The control frequency was 4.83% in 1,076 eggs counted. These investigators performed the experiment according to the procedure described by Shankaranarayanan (1967) and determined the statistical significance to be at the 5% level, although they did not mention the type of statistical test employed. Based on these observations, this study is evaluated as an indicator of a positive response. A comparable study in an independent laboratory would be of interest for comparative purposes.

Inoue and Watanabe (1978) studied the effects of cadmium chloride in the sex-linked recessive lethal test (attached-X method) in *Drosophila melanogaster*, Oregon-R flies. In this test, the induction of mutations is measured by the reduction in the proportion of males. The sex ratio (0.528) in the experimental group treated with 50 ppm (2.72×10^{-4}M) was not statistically different from the sex ratio of controls (0.54), indicating cadmium chloride is nonmutagenic. The dosage selected was a maximally tolerated dose. Both positive (AF-2) and negative controls were used in the experiment.

Ramel and Magnusson (1979) failed to detect nondisjunction and sex chromosome loss in *Drosophila* following treatment of larvae with 62 ppm (3.32×10^{-4}M) of cadmium chloride. No data were presented; therefore, this study cannot be evaluated.

Chromosomal aberrations were observed in the testes of the grasshopper, *Poekilocerus pictus*, injected abdominally with 0.001 (5.45×10^{-9}M), 0.01 (5.45×10^{-9}M), and 0.05% (2.27×10^{-7}M) cadmium chloride in 0.05 ml volumes (Kumaraswamy and Rajasekarasetty 1978). Stickiness of chromosomes, bridge formation at anaphase-I, and tetraploidy at metaphase were noted. The test cannot be considered adequate because no controls were used and no tabulated data were presented. The possibility of technical artifacts must also be considered, particularly because chromosomal preparations were made by a squash technique, and there were no controls.

CHROMOSOMAL ABERRATIONS IN HUMAN AND OTHER MAMMALIAN SYSTEMS

Chromosomal damage studies of cadmium, both *in vitro* and *in vivo*, are summarized in Tables 4 and 5. A discussion of each study follows.

TABLE 4. MUTAGENICITY EVALUATION OF CADMIUM: *IN VITRO* CHROMOSOMAL ABERRATIONS

Test System	Duration of Cultures	Cadmium Compound	Dosage	Duration of Treatment	Activation System	Results	Comments	Reference
Human blood lymphocytes	72 hrs	Cadmium sulfide (solvent not specified)	6.2×10^{-2} ug/ml	4 hrs 8 hrs	None	Reported as positive	1. Blood lymphocytes were derived from only one individual. 2. Only 50 metaphases for each end point were scored. 3. Only one concentration of the test compound was used.	Shiraishi et al. (1972)
Human blood lymphocytes	48 hrs 72 hrs	Cadmium chloride aqueous solution	5×10^{-5}M 5×10^{-6}M	24 hrs 48 hrs 72 hrs	None	Reported as negative	1. Toxicity was determined. 2. Appropriate dosages used. 3. 100 metaphases scored for each point.	Dekundt and Deminatti (1978)
Human blood lymphocytes	48 hrs	Cadmium chloride aqueous solution		48 hrs	None	Reported as negative	1. Data are not provided. 2. Concentrations of the test compound not specified.	Paton and Allison (1972)
Cell line WI38 and MCR5	24 hrs			24 hrs		Reported as negative		
Human blood lymphocytes G_0 stage	48 hrs	Cadmium acetate aqueous solution	10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4}M	3 hrs	None	Reported as weakly positive	1. No dose-response. 2. Experiments were not repeated to confirm the positive finding.	Gasiorek and Bauchinger (1981)

(continued on the following page)

TABLE 4. (continued)

Test System	Duration of Cultures	Cadmium Compound	Dosage	Duration of Treatment	Activation System	Results	Comments	Reference
Chinese hamster "Hy" cell line		Cadmium sulfate aqueous solution	10^{-4}M	1 hr and harvested at 2,4,6,8, 10,12,15,18, 21,24,27,30, days	None	Reported as positive	1. Colchicine-like effect 2. The type of sera has not been specified. 3. Protocol for chromosome preparation has not been specified.	Rohr and Bauchinger (1976)
Chinese hamster CHO cell line		Cadmium chloride in 0.1 MHCl	2×10^{-6}M	12, 24, 36, and 48 hrs	None	Reported as positive only in the presence of newborn calf (bovine) or human serum. Negative in the presence of fetal calf serum.	1. Threshold dosage was established as 1×10^{-1}M for chromosomal aberration with newborn calf and human sera. 2. Classificaton of aberration types not given. 3. Active only in the presence of fetal calf serum.	Deaven and Campbell (1980)
Mouse Mammary Carcinoma FM3A		Cadmium chloride aqueous solution	6.4×10^{-5}M 3.2×10^{-5}M 1.0×10^{-5}M	24 and 48 hrs 24 and 48 hrs 24 and 48 hrs	None	Reported as negative	1. 6.4×10^{-5} too toxic. 2. Experiments were repeated to confirm the results.	Umeda and Nishimura (1979)

TABLE 5. MUTAGENICITY EVALUATION OF CADMIUM: IN VIVO CHROMOSOMAL ABERRATIONS IN HUMANS

Species	Number of Exposed Workers	Number of Controls	Duration of Exposure	Duration of Culture (hrs)	Number of Metaphases Analyzed	Results	Comment	Reference
Human blood lymphocytes	14	5	3 months-26 years	48	2800 (exp) 900 (control)	Reported as negative	1. Sample size too small.	Dekundt et al. (1973)
Human blood lymphocytes	40	13	6 weeks-34 years	48	3740 (exp) 1243 (control)	Reported as negative	1. Study has been conducted following good cytogenetic procedure.	O'Riordan et al. (1978)
Human blood lymphocytes	7, 12	6, 9	Not given	72	155/ person	Reported as positive	1. The history of the patients including exposure to other drugs was not given in this paper.	Shiraishi and Yoshida 1972 Shiraishi 1975
Human blood lymphocytes from cadmium-exposed workers	5	3	5-24 years	48-72	100/ person	Reported as negative	1. Sample size too small.	Bui et al. (1975)
Itai-Itai patients blood lymphocytes	4	4		72	100/ person	Reported as negative		
Human blood lymphocytes	24	15	3-6.5 years	48	4800 (exp) 1650 (control)	Reported as positive	1. The possiblity of synergistic action of various metals cannot be ignored.	Rauchinger et al. (1976)

(continued on the following page)

TABLE 5. (continued)

Species	Source of Cells	Cadmium Compound	Dosage	Duration of Treatment	Results	Comments	Reference
Mouse bone marrow	Bone marrow cells	Cadmium chloride	0.06% in diet	30 days	Reported as negative	1. Good technical procedure. 2. Data were analyzed statistically.	Dekundt and Gerber (1979)
Mouse Micronucleus	Bone marrow cells	Cadmium chloride	4 mg/kg/ day	5 days	Reported as negative	1. Number of mice per group was 3. 2. Number of polychromatic erythrocytes scored was 300 from each mouse.	Heddle and Bruce (1977)
Rat Embryos	Embryonic cells	Kilhman virus Cadmium chloride			Reported as positive	1. Cadmium enhanced the effects of virus. 2. Cadmium alone was ineffective.	Zasukhina et al. (1977)
		Cadmium chloride only			Reported as negative		
Mouse Dominant lethals	Score dead proportion of implants in the uterus	Cadmium chloride	1.35, 2.7, 5.4, mg/kg	1 day	Reported as negative	1. Standard dominant lethal assay was performed. 2. The entire spermatogenic cycle was covered.	Epstein et al. (1972)

(continued on the following page)

TABLE 5. (continued)

Species	Source of Cells	Cadmium Compound	Dosage	Duration of Treatment	Results	Comments	Reference
Mice Dominant lethals	Score dead proportional implants in the uterus	Cadmium chloride	1.75 mg/kg	1 day	Reported as negative	1. All cell stages not sampled.	Gilliavod and Leonard (1975)
Mice (female) Dominant lethals	Score dead and live implants in the uterus	Cadmium chloride	2 mg/kg	0.5 to 4.5 days	Reported as negative	1. Experiments repeated three times.	Suter (1975)
Mice Heritable Translocation	Testicular cells from F_1 males	Cadmium chloride	1.75 mg/kg	1 day	Reported as negative		Gilliavod and Leonard (1975)
Mice (female)	Oocytes	Cadmium chloride	3 mg/kg 6 mg/kg	12 hrs	Reported as positive	1. Technical difficulties in processing oocytes not discussed.	Shimada et al. (1976)
Syrian hamsters (female)	Oocytes	Cadmium chloride			Reported as positive	1. Technical problems in processing oocytes not not discussed.	Watanabe et al. (1979)
Mice (male)	Spermatocytes	Cadmium chloride	0.5, 1.75 3.0 mg/kg	90 days	Reported as negative		Gilliavod and Leonard (1975)

Studies on Human Chromosomes In Vitro

Shiraishi et al. (1972) tested cadmium sulfide for the induction of chromosomal aberrations in cultured human blood lymphocytes. Lymphocytes from a normal human female were cultured for 72 hours at 37°C. Eight and four hours prior to harvesting, the cultures were treated with cadmium sulfide at a concentration of 6.2×10^{-2} ug/ml of culture fluid. Control cultures were incubated similarly without the addition of cadmium sulfide. Three hours prior to harvesting, cells were treated with 0.02 ug/ml of colcemid to obtain cells in the metaphase stage of mitosis. Chromosome preparations were made with the standard procedure (air-drying technique) and stained with Giemsa stain. Fifty metaphase cells were scored from each treatment group for chromosome aberrations. The types of aberrations described include chromatid and isochromatid breaks, and symmetrical and asymmetrical translocations. Increased incidences of chromosomal aberrations, 52% in the 4-hour treatment group and 60% in the 8-hour treatment group, were noted over the control value of 0%. This study utilized a blood sample from only one donor; the history of the donor was not discussed. Only one concentration of the compound was used, and hence, no dose-response relationship is available. No information was given on the solvent used to dissolve the test compound, and the number of cells scored was small. No indication as to the reproducibility of results was given, and therefore, this study cannot be regarded as strong evidence for a cytogenetic effect of cadmium.

Dekundt and Deminatti (1978) investigated the mutagenic effects of cadmium chloride in cultured human lymphocytes. They treated two batches of cell cultures and analyzed chromosomes as follows: One batch of cultures was treated at 0 hour and at 24 hours after the initiation of cell cultures with 5×10^{-5} and 5×10^{-6}M cadmium chloride. Chromosome preparations were made

48 hours after the initiation of the culture using the standard air-drying technique. In cultures treated 0 hour after the initiation, one hundred metaphases were scored for each dosage. There were 3% aberrations (1% aneuploidy, 2% gaps) at 5 x 10^{-5}M and 7% aberrations (5% aneuploidy, 2% gaps) at 5 x 10^{-6}M. In cultures treated 24 hours after the initiation of cultures, there were 5% aberrations (1% aneuploidy, 4% gaps) at 5 x 10^{-5}M and 2% aberrations (1% gaps and 1% fragments) at 5 x 10^{-6}M. The control aberration frequency was 5% (3% aneuploidy, 2% gaps). The other batch of cultures was treated at 0 hour and 24 hours, and chromosome preparations were made 72 hours after the initiation of cell cultures. One hundred metaphases were analyzed for aberration frequencies from each group. In cultures treated at 0 hour, there were 4% aberrations (3% aneuploidy, 1% gaps) at 5 x 10^{-5}M and 3% aberrations (3% aneuploidy) at 5 x 10^{-6}M. Cultures treated after 24 hours of initiation exhibited 6% aberrations (2% aneuploidy, 1% fragment, 3% gaps) at 5 x 10^{-5}M and 4% aberrations (1% aneuploidy, 2% gaps) at 5 x 10^{-6}M. The control frequencies were 1% aneuploidy and 1% gaps. The first batch of cultures exhibited aberration frequencies similar to the control level. The second batch of cultures, treated only 24 hours after the initiation, exhibited aberration frequencies two to three times above the control level. These aberrations were mostly in the form of aneuploidy and gaps. The significance of chromosomal gaps is not yet understood and they may not represent true chromosomal aberrations because they have a tendency of restitution. Furthermore, the slight increase in the incidence of aneuploidy may be due to technical difficulties, such as scattering of chromosomes while preparing slides, leaving uneven numbers. These results may be treated as a negative response of cadmium in inducing chromosomal aberrations.

Paton and Allison (1972) exposed human lymphocyte cultures and cultures of the established human cell lines WI38 and MRC5 to at least two concentrations (not specified) of cadmium chloride. The duration of treatment was 48 hours for lymphocytes and 24 hours for WI38 and MRC5. Chromosomal preparations from 100-200 cells were analyzed for aberrations. No aberrations were recorded in treated cells, but data are not given. In the absence of details of concentrations and data, this study cannot be critically evaluated.

Gasiorek and Bauchinger (1981) exposed unstimulated human blood lymphocytes (G_0) in 1 ml quantities to 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}M of cadmium acetate for 3 hours. The cells were washed free of cadmium acetate and grown in medium containing fetal calf serum and PHA for 48 hours at 37°C; chromosome preparations were made with the standard air-drying technique. Chromosome analysis from 200 cells per treatment indicated a dose-related increase in the incidence of chromosome gaps. The frequencies of gaps were 0.160, 0.115, 0.135, 0.085, and 0.055 per cell, respectively, for the above doses compared to the control frequency of 0.058 per cell. Data were analyzed by the Mann-Whitney rank U-test to compare the incidence of chromosome changes in different samples (significance taken as $P < 0.05$). The frequencies of structural aberrations (chromatid deletions and acentric fragments) were 0.025, 0.010, 0.005, 0.020, and 0.010 per cell, respectively, for the same doses, whereas in controls the frequency of structural aberrations was 0.005 ± 0.005 per cell. Analysis by Mann-Whitney rank U-test indicated that structural chromosome aberrations were significantly higher than the controls, although no dose-response relationship was evident. No metabolic activation system was used. Sufficient number of metaphases (200 per dose) was scored and a standard protocol was employed. Although these suggest a mutagenic response, the lack of a dose-dependent response makes it important that the experiments be confirmed in another study.

Studies on Rodent Chromosomes In Vitro

Rohr and Bauchinger (1976) studied the effects of cadmium sulfate in the Chinese hamster cell line "Hy" using three types of experiments. In a long-term experiment without recovery, cells were exposed to cadmium sulfate at concentrations from of 10^{-8} to 10^{-5}M. Chromosome preparations were made following treatment of cells for 16 hours with 0.2 ug/ml of colcemid and hypotonic solution. The 16-hour time period was chosen in order to analyze the cells after exposure during a whole cell cycle. Concentrations of 10^{-5}M were toxic to cells after 16 hours of exposure, and hence, chromosome analysis could not be made. In a short-term experiment without recovery, cells were treated only for 3 hours at a concentration range of 10^{-8} to 10^{-4} mol and chromosome preparations were made without the addition of colcemid and hypotonic solution. This experiment indicated a typical stathmokinetic effect (spindle inhibition) similar to that caused by colcemid. The mitotic index increased with higher concentrations of cadmium sulfate. In a short-term experiment with recovery, a concentration of 10^{-4}M was chosen and cells grown on coverslips were exposed for 1 hour. Cells with coverslips were washed free of cadmium sulfate and transfered to fresh medium and grown for 2 to 33 hours. Chromosome preparations were made at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, 30, and 33 hours after the cells were transferred to the test medium. A total of 500 cells were scored for each recovery period. The incidences of aberrations (0.2 to 0.6% structural and 2.4 to 3.7% numerical) after 2 to 12 hours of recovery were similar to those of control levels (0.1% structural and 2.4% numerical). Between 15 and 21 hours, the structural aberrations ranged from 10.2% to 22.8% and the numerical aberrations ranged from 3.0% to 4.9%. From 24 to 33 hours, the aberration frequencies were lower compared to the interval of 15 and 21 hours. During this period, the structural aberrations ranged from 1.2 to 4.4% and the numerical aberrations ranged from 7.8% to 10.8%. Only one dose was used in this study. A common control was maintained for all these intervals, which may not be satisfactory. The exact type of serum used in this study was not indicated and the type of serum can influence the yield of aberrations.

Deaven and Campbell (1980) studied the effects of cadmium chloride on chromosomes in CHO cells grown in the presence of bovine serum and fetal calf serum. A concentration of 2 x 10^{-6}M cadmium produced 17, 26, 62, and 74% damaged cells, respectively, at 12-, 24-, 36-, and 48-hour analyses of metaphase chromosomes. However, the presence of fetal calf serum and 2 x 10^{-6}M cadmium chloride did not induce growth inhibition or chromosome aberrations. According to these investigators, fetal calf serum appears to protect the cells from the damaging effects of cadmium, whereas newborn calf serum and human serum actively transport cadmium ions into the nucleus, thus damaging the chromosomes. These authors also examined the frequencies of sister chromatid exchanges (SCEs) in cells grown in F-10 containing 15% newborn calf serum at a concentration of 4 x 10^{-7}M cadmium chloride (low, marginal toxicity). The SCE rate was not elevated above control levels (10 SCEs/cell). The range was 2 to 18 for cadmium-treated cells and the range for controls was 4 to 19 per cell. This report is assessed as inconclusive because more information on the exact role of serum in causing chromosome aberrations is still not known.

Umeda and Nishimura (1979) investigated the clastogenic effects of cadmium chloride in FM3A cells derived from C3H mouse carcinoma. Cells were grown in Eagles minimal essential medium supplemented with 10% bovine serum. Cells were exposed to 6.4×10^{-5}, 3.2×10^{-5}, and 1.0×10^{-5}M of aqueous cadmium chloride. After 24 and 45 hours of exposure, chromosome preparations were made and analyzed. One hundred metaphases were scored for each dose. No significant increase in the aberration frequency was noted in treated cultures as compared to control cultures. There were no metaphases in cells treated with 6.4×10^{-5}M either at 24 hours or at 48 hours, indicating toxicity. At 3.2×10^{-5}M the aberration frequencies were 2% and 3%, at 24 and 48 hours respectively. At the lowest concentration of 1.0×10^{-5}, the aberration frequencies were 1%, each for the 24- and 48-hour treatment. The control cultures exhibited 2% aberrations at 24 hours and 1% aberrations at 48 hours. Experiments were performed using the accepted procedures. Three concentrations of the test compound were used and 100 metaphases were scored for evaluation. This report is assessed as a negative response of cadmium in inducing chromsomal aberrations.

Zasukhina et al. (1977) reported increased aberration yields in rat embryos exposed to virus and cadmium chloride. They infected rat embryo cultures with Kilhman virus and then introduced cadmium chloride (3.5×10^{-6}M) into the cell cultures. Chromosome preparations were performed 24 hours after the infection. Examination of metaphase cells revealed 10% aberrations as compared to the control value of 2% aberrations. In control cultures infected with virus only, the aberration frequency was 6%, and in cultures treated with cadmium chloride only, there were 3% aberrations. These results indicate that cadmium chloride may enhance virus-induced chromosomal aberrations but does not induce chromosomal aberrations by itself.

Studies on Human Chromosomes In Vivo

Shiraishi and Yoshida (1972) and Shiraishi (1975) obtained markedly positive results from Japanese Itai-Itai patients. The Itai-Itai disease is believed to have been induced by cadmium contamination. Analysis of blood lymphocytes from 72-hour cultures derived from these patients exhibited a high degree of chromosomal aberrations (26.7%) compared to the control aberration rate (2.6%). Blood cadmium level was not given in this paper. See Table 5 for exposure parameters.

The results of Shiraishi and Yoshida (1972) and Shiraishi (1975), were contradictory to the results of Bui et al. (1975) who performed chromosomal analysis in four Itai-Itai patients (blood cadmium level 15.5-28.8 ng/g), five Swedish workers exposed to cadmium (blood cadmium level 24.7-61 ng/g), four Japanese controls (blood cadmium level 4.4-6.1 ng/g), and three Swedish controls (blood cadmium level 1.4-3.2 ng/g). The incidences of aberrations after 72 hours of culture were 2.3% numerical aberrations and 6.6% structural aberrations in the Itai-Itai patients as compared to the Japanese controls having frequencies of 4.5% numerical and 6.0% structural aberrations, indicating there was no difference between the controls and Itai-Itai patients. In the five Swedish workers exposed to cadmium, the incidences of chromosomal aberrations were 1.0% numerical and 2.0% structural aberrations, while in the three Swedish controls the frequencies were 0.7% numerical and 4.7% structural aberrations indicating nonmutagenic response.

The reason for the discrepancy between the results of Shirashi and Yoshida (1972) and Bui et al. (1975) in Itai-Itai patients could possibly be due to some other factor, such as the time of initiation of cultures after the blood was drawn. In the experiment of Bui et al., the subjects were not exposed to drugs and x-rays, nor did they suffer from viral infections at the time of venepuncture. These factors were not controlled for in the study by Shirashi and Yoshida.

Dekundt et al. (1973) investigated the incidence of chromosome aberrations in 14 workers exposed to zinc, lead, and cadmium in a zinc smelting plant. The workers were classified into three groups based on the degree of exposure. Group 1 consisted of five workers exposed to high levels of zinc (concentration not specified), low levels of lead (1% w/w of the mineral), and cadmium (concentration negligible). Group 2 consisted of five workers exposed to dust with high levels of all three metals: zinc (concentration not specified), lead (4% w/w), and cadmium (1% w/w). Group 3 consisted of four workers exposed to mud and dust containing high levels of lead (60% w/w) and cadmium (1% w/w). The control group consisted of three normal (control) individuals. Chromosomal analysis from blood lymphocytes cultured for 72 hours indicated 3.87%, 1.6%, and 2.76% aberrant cells, respectively, in groups 1, 2, and 3, while the control frequency was 1.55%. Since the incidence of aberrations in group 3 was less than the incidence of aberrations in group 1, it does not appear that cadimum enhanced the aberration frequency in this study. In addition, analysis of their data using the t-test indicated that cadmium exposure did not induce a significant increase in the aberration frequency. The blood cadmium level was not determined in this experiment.

Bauchinger et al. (1976) studied 24 workers (25-53 years of age) exposed to lead (mean blood lead level 1 ± 7 ug/100 ml) and cadmium (mean blood cadmium level 0.40 ± 0.27 ug/ml). The workers were exposed to these metals for approximately 3 to 6.5 years at a smelting plant. Of the 4,800 metaphases scored from lymphocytes cultured for 48 hours, an increase in both chromosome and chromatid type aberrations (1.354 ± 0.994%) was noted compared to a frequency of 0.467 ± 0.916% aberrations in 1,650 metaphases derived from 15 controls (mean blood cadmium level 0.15 ug/ml). The authors point out that "the observed chromosome aberrations cannot be causally related to cadmium because the workers were also exposed to lead and zinc." Dekundt and Leonard (1975) reported a significant ($P < 0.02$) increase in the incidence of "complex chromosomal aberrations" in a group of 23 men exposed to high levels of cadmium and lead (23.5 to 75.9 ug/100 ml) compared to controls.

O'Riordan et al. (1978) studied chromosomal aberrations in blood lymphocytes from 40 workers exposed to cadmium salts, (name not specified, mean blood cadmium level 1.95 ug/100 ml range < 0.2 to 14.0 ug/100 ml) for a period of 6 weeks to 34 years. In 3,740 cells examined from these workers, there were four chromatid interchanges. In the control population of 1,243 cells derived from 13 normal subjects (mean blood cadmium level less than 0.2 ug/100 ml in 8 donors and 0.6 to 2.9 ug/ml in 5 donors), there were no aberrations at all. Data were pooled from all 40 workers. It is not clear whether the four chromatid interchanges came from one exposed worker or more than one worker. The occurrence of chromatid exchanges though small in number, 4/3,740 cells, does not necessarily indicate a negative response but does indicate that the study may be considered inconclusive.

Most of these studies on workers reflect mixed exposure to other metals such as zinc and lead. Since smelters also commonly process relatively crude materials, exposure to other metals such as chromium, nickel, etc., cannot be eliminated as possible contributors to the observed effect. Synergistic effects may also confuse the results.

Studies on Rodent Chromosomes In Vivo

Dekundt and Gerber (1979) investigated the in vivo cytogenetic effects of cadmium chloride (3.27×10^{-7}M, 0.06%) in mice. Mice were maintained on a standard diet (1.1% calcium) or on a low calcium diet (0.03%) for one month. In both cases the diet was supplemented with cadmium chloride. Cadmium is known to be absorbed through the gut. Cadmium chloride did not induce chromosome aberrations in bone marrow cells significantly above the control level either in normal or low calcium diet groups. The frequency of aberrations in animals treated with cadmium chloride in the standard diet (1.1% calcium) was 2.20%, and the frequency of aberrations in animals treated with cadmium chloride in the low calcium diet (0.03%) was 1.60%. The control frequencies were 1.8% and 2.0%, respectively. These results indicate that cadmium chloride does not induce chromosomal aberrations in mice.

Micronucleus Assay

The micronucleus assay is based on the observation that chromosome fragments are produced by mutagenic chemicals. Those fragments lacking centromeres are unable to segregate normally but lag behind during the cell division to form small nuclei or micronuclei in daughter cells. Heddle and Bruce (1977) studied the ability of cadmium chloride to induce micronuclei in the mouse. Three groups of mice (F_1 of C57Bl/6X C3H/He), each group containing three animals, were given daily intraperitoneal injections of cadmium chloride for 5 days with total doses of 1, 6, and 16 mg/kg, respectively. Mice were sacrificed, bone marrow smears were prepared, and 333 polychromatic erythrocytes from each mice were scored for the presence of micronuclei. No increase in the incidence of micronuclei was observed. This report deals with three mice in each group and only 333 cells from each mouse; thus, a total of 1,000 cells were analyzed for each dose group. The spontaneous frequency of micronuclei was 0.5%. An observation of 1% over the control value was considered a positive response. According to these authors, the frequency of micronuclei in the experimental groups was not different from the control level. Confirmation of the results are required using a larger sample of animals (10) per dose group and an analysis of at least 2,000 polychromatic erythrocytes per dose group. Consequently, this report is regarded as inconclusive.

Dominant Lethal Assay

The ability of cadmium chloride to induce dominant lethal mutations, which result in the death of fetuses during various stages of development, has been investigated (Epstein et al. 1972; Gilliavod and Leonard 1975; Ramaiya and Pomerantseva 1977; Suter 1975; Sutou et al. 1980 a, b).

Epstein et al. (1972) evaluated the dominant lethal effects of cadmium chloride in ICR/Ha mice. Groups of seven or nine male mice, 8 to 10 weeks of age, were injected intraperitoneally with 1.35, 2.7, 5.4, and 7.0 mg/kg of cadmium chloride in distilled water. Treated males were bred with virgin females, 8 to 10 weeks of age. Each male was allowed to mate with three virgin females per week for 8 weeks. Mated females were sacrificed on the 13th day and analyzed for dead (dominant lethals) and live implants. According to these authors, cadmium chloride did not induce a statistically significant increase in dominant lethal mutations over the control value.

Gilliavod and Leonard (1975) investigated the dominant lethal effects of cadmium chloride in another strain of mice BALB/c. Only one dose of 1.75 mg/kg cadmium chloride was injected into male mice (11-13 weeks of age) through the intraperitoneal route. The treated males were bred with three virgin females every week for 3 weeks. The mated females were sacrificed on the 10th day and the number of corpora lutea, and dead and live implants were counted and compared with the controls. No dominant lethal effect was observed in treated and control groups. These investigators treated the parental male mice with only one acute dose of the test compound.

Furthermore, they bred the treated mates with normal females for only 3 weeks, which is too short to sample stages of spermatogenesis. The standard way of doing an dominant lethal test is to breed the treated males for 8 weeks. Consequently, this report is treated as inconclusive.

Suter (1975) studied the mutagenic effects of cadmium chloride using the dominant lethal assay in female mice (F1 progeny of C_3H and C57BLA). According to this investigator cadmium chloride had no dominant lethal effects in female mice. Female mice, F_1 (10 x C_3H) stock, were injected intraperitoneally with 2 mg/kg cadmium chloride and bred with untreated males 0.5 to 4.5 days postinjection. Mated females, as evidenced by the vaginal plug, were sacrificed 12-15 days later and the number of corpora lutea, number of total implants, number of living implants, and the percent of dead implants per female were counted. No differences were noted between the treated and control groups. In the treated group, the frequency of corpora lutea, total implants, living implants, and dead implants per female were 8.2, 7.8, 6.9, and 6.9, respectively, as compared to control frequencies of 7.6, 6.8, 6.4, and 6.1% per female.

Ramaiya and Pomerantseva (1977) investigated the mutagenic effect of cadmium chloride using the dominant lethal test. F_1 hybrid mice (CBA x C57BL) aged 2.5 to 3 months, were selected for these studies. Males were given a single intraperitoneal injection of aqueous cadmium chloride solution. Three doses, 1.0, 2.0, and 4 mg/kg, were employed. LD_{50} was determined to be 6.9 mg/kg. Treated males were mated with untreated females for a period of 6 weeks covering the entire spermatogenic cycle. Dominant lethals, as noted by preimplantation and postimplantation losses and the ratio between the dead and live implants, were recorded. No significant ($P > 0.01$) increase in the dominant lethal frequency was recorded. These results are regarded as negative since the authors followed appropriate protocols, the dosage selection was based on LD_{50}, and data were analyzed statistically.

From these studies it appears that cadmium chloride has no mutagenic potential in the mammalian dominant lethal test. The dominant lethal test has been extensively used in screening for the mutgenic activity of chemicals.

Heritable Translocation Assay

Gilliavod and Leonard (1975) evaluated the mutagenic effects of cadmium chloride in BALB/c mice using the F_1 heritable translocation assay. Male mice (number not specified) were treated with 1.75 mg/kg of cadmium chloride intraperitoneally and each treated male was bred with three nontreated virgin females each week for 3 weeks. The spermatocytes of the resulting 120 F_1 male progeny were analyzed for the presence of heritable chromosomal translocation by standard cytogenetic methods. No evidence of heritable translocation was noted in the spermatocytes of F_1 males. Only a single concentration was used in this experiment. Mating of treated males was done only for 3 weeks instead of 8 weeks. No controls were maintained. Consequently, this report is treated as inconclusive.

Direct Effects on Germ Cells

Effects of cadmium chloride on oocytes of mice (Shimada et al. 1976), Syrian hamsters (Watanabe et al. 1979), and on spermatocytes of mice (Gilliavod and Leonard 1975) have been investigated.

Shimada et al. (1976) induced superovulation by injecting female mice, ddy strain, with 5 international units (I.U.) of pregnant mare's serum (PMS) followed 48 hours later by 5 I.U. of human chorionic gonadotrophin (HCG). Mice were given 3 mg/kg or 6 mg/kg of cadmium chloride 3 hours after the administration of HCG and were dissected 12 hours after the cadmium chloride administration. Chromosome preparations were made from unfertilized oocytes at the second meiotic metaphase as described by Tarkowski (1966). No structural chromosome abnormalities were found. However, numerical aberrations (aneuploidy) were found to be statistically significant ($P = 0.015$) in the dose group of 3 mg/kg group as compared to controls. The authors postulated that this nondisjunction may be due to the spindle inhibiting effects of cadmium.

Watanabe et al. (1979), using Syrian hamster oocytes and cadmium chloride, reported even more pronounced incidence of aneuploidy. Cadmium chloride at concentrations of 1.0, 2.0, and 4 mg/kg were subcutaneously injected to groups of 20 female Syrian hamsters 5 hours before ovulation. Matched controls were given equal volume of normal saline. Females were sacrificed 12 hours after the treatment, and the oocytes were recovered from the ampulla. Analyses revealed that 6 females out of 20 from the 1.0 mg/kg group, 11 females out of 20 from the 2.0 mg/kg group, and 12 females out of 20 from the 4.0 mg/kg group had numerical chromosomal abnormalities, such as hyperhaploidy and diploidy, in oocytes as compared to 3 out of 20 in control females. The results were statistically significant ($P < 0.05$ and $P < 0.01$) in the treated groups compared to the control group. Cadmium-treated animals were also analyzed for cadmium accumulation in the ovary using atomic absorption spectrophotometry. The results indicated statistically significant ($P < 0.05$) increases in the accumulation of cadmium in the ovaries of treated females as compared to control females. Both of these results appear to indicate a positive response of cadmium in inducing numerical chromosomal abnormalities in mammalian oocytes.

Gilliavod and Leonard (1975) investigated the mutagenic effects of cadmium chloride in BALB/c mice using the spermatocyte assay. Males in groups of 10 were treated with 0.5, 1.75, and 3.0 mg/kg of cadmium chloride intraperitoneally. After 3 months, treated males were sacrificed and spermatocytes (100 cells per animal) in the testes were analyzed for translocations that may have been passed on from treated spermatogonia. No translocations were found, i.e., the frequency of translocations were 0 in both the treated and control animals. This is not a very sensitive test, and hence, it is not commonly employed in mutagenicity tests.

Sperm Abnormality Assay in Mammals

Heddle and Bruce (1977) evaluated the effects of cadmium using the sperm abnormality assay. The sperm abnormality assay is based on the observation of increased incidence of sperm heads with abnormal shapes as a result of exposure to chemical mutagens (Wyrobek and Bruce 1975). Three groups of mice of the genotype (C57BL/6 x C3H/He)F_1, each consisting of three mice, were given daily intraperitoneal injections of cadmium chloride for 5 days with doses of 1, 4, and 16 mg/kg, respectively. Sperm suspensions were made from sperm collected from the cauda epididymis following the sacrifice by cervical dislocation of mice. The sperm suspension was stained with 1% eosin-Y in water, and smears were made, dried, and mounted under a coverslip with permount. One thousand sperm heads were evaluated for morphological abnormalities. The background frequency of sperm head abnormality in the control population was 1%. Under the conditions of the test, there were no increases in sperm head abnormalities in the treated over the control groups.

CHROMOSOMAL ABERRATIONS IN PLANTS

Levan (1945) reported that treatment of Allium cepa root-tips with cadmium chloride induced C-mitosis. This observation was later confirmed by Avanzi (1950) using cadmium chloride concentrations ranging from 2 x 10^{-6}M to 5 x 10^{-2}M. Oehlkers (1953) reported that cadmium nitrate induced chromosomal aberrations in Vicia faba. Van Rosen (1953, 1954) reported the genotoxicity of cadmium as evidenced by chromosomal aberrations in the root-tips of plants such as Allium cepa, Beta vulgaris, Pisum abysinnicum, and Vicia sativa. Similar observations were made by Degraeve (1971) in Horedeum sativum and by Ruposhev and Garina (1977) in Crepis capillaris. Aberrations reported in these studies were of both chromatid and chromosome type with a dose-related response. Since many of these publications are in foreign languages, the material presented here is a summary derived from the review article published by Degraeve (1981).

OTHER INDIRECT EVIDENCE

Some information is available on the effects of cadmium on animals, and although it is not strictly mutagenicity test data, it may be useful in evaluating the ability of cadmium to reach and damage the gonads. Dixon et al. (1976) reported that cadmium chloride at 2.24 mg/kg administered intraperitoneally caused damage to rat testes. A single 10 mg/kg intraperitoneal injection causes selective destruction of rat testes. Cadmium chloride, when administered intraperitoneally at 1 mg/kg, reduced fertility of male mice at sperm cell stages, except spermatozoa (Lee and Dixon 1973). However, single oral doses up to 25 mg/kg had no effect on the fertility of male rats (Dixon et al. 1976), and cadmium chloride at 0.1 mg/l in drinking water for up to 90 days had no effect on the fertility of rats.

Intraperitoneal injection of 1 mg/kg, cadmium chloride decreased the incorporation of thymidine into spermatogonia in mice (Lee and Dixon 1973). These authors (Lee and Dixon 1973) also found the binding of cadmium to late spermatids *in vivo* or *in vitro*. Friedman and Staub (1976) studied the effects of cadmium chloride on DNA synthesis in Swiss mice. Cadmium chloride at 10 mg/kg inhibited DNA synthesis significantly. An aqueous solution of cadmium chloride was injected intraperitoneally at the above dose into five male mice and the mice were sacrificed 3.5 hours later. Thirty minutes prior to sacrifice, mice were injected with 10 uCi [^{3}H] thymidine. Controls received only 10 uCi [^{3}H] thymidine. Testes were removed following cervical dislocation, DNA was isolated, and the specific activity was determined. Cadmium chloride induced a statistically significant ($P < 0.01$) inhibition of [^{3}H] thymidine uptake (1.90 ± 0.58) in the testes as compared to controls (7.45 ± 1.44).

Mitra and Bernstein (1977, 1978) reported that when *E. coli* cultures were exposed to 3×10^{-6}M cadmium (Cd^{2+}), 82 to 95% of the cells lost their ability to form colonies on agar plate. Analysis of DNA strands from cells treated with various doses of Cd^{2+} indicated that there was a dose-related increase in the breakage of single strand DNA. These investigators believe that the loss of viability in cadmium-treated cells is due to the single-strand DNA breakage. Cadmium-treated cells recovered viability when grown in Cd^{2+}-free liquid medium containing 10 mM hydroxyurea.

Sirover and Loeb (1976) investigated the infidelity of DNA synthesis brought about by cadmium chloride and cadmium acetate. This assay measures the perturbations in the fidelity of DNA synthesis *in vitro* caused by soluble metal salts. Cadmium chloride and cadmium acetate decreased the fidelity of DNA synthesis. Cadmium chloride also induced concentration-dependent inhibition of RNA synthesis (Hoffman and Niyogi 1977).

SUMMARY

Cadmium has been investigated for mutagenic activity in both prokaryotic and eukaryotic systems. Gene mutation studies in Salmonella typhimurium and E. coli have produced inconclusive results. In yeast, gene mutation studies are also inconclusive. However, gene mutation studies in mammalian cell cultures, mouse lymphoma cells, and Chinese hamster lung and ovary cells, have resulted in a weak mutagenic response. Rec-assay, which is a test for DNA repair in Bacillus subtilis, also resulted in a weak mutagenic response. In the Drosophila sex-linked recessive lethal test, cadmium has been found to be nonmutagenic. However, the negative response may be due to inadequate test control. In contrast, the dominant lethal test in Drosophila resulted in a positive response with a dose-response relationship. Chromosomal aberration studies in human lymphocytes and human cell lines treated with cadmium are conflicting and contradictory. In Chinese hamster cells, chromosomal aberrations were noted following treatment with cadmium, however, in mouse carcinoma cells no aberrations were recorded in response to cadmium treatment. In rodents, treatment with cadmium induced no chromosomal aberrations or micronuclei in bone marrow cells. Similarly, no dominant lethal mutations were noted in mice treated with cadmium. Induction of chromosomal nondisjunction in female germ cells of mice and Syrian hamsters by cadmium has been reported. Chromosomal aberrations and gene mutations in plants exposed to cadmium have also been recorded.

A clear analysis of the mutagenicity of cadmium in the studies discussed herein is rather difficult because of the conflicting results and lack of adequate test protocols used. However, the results of gene mutations studies in mammalian cell cultures, rec-assays in bacteria, chromosomal nondisjunction studies in intact mammals, as well as other indirect evidences suggest that cadmium is weakly mutagenic.

CARCINOGENICITY

The topic of the carcinogenicity of cadmium has been reviewed critically in earlier documents (IARC 1973, 1976; U.S. EPA 1977, 1981; Sunderman 1977, 1978; Hernberg 1977). This section updates findings mentioned previously and discusses recent findings not mentioned in earlier reviews.

ANIMAL STUDIES

Injection Studies in Mice and Rats

Injection of cadmium metal or certain salts of cadmium has been shown to produce sarcomas at the site of the injection as well as testicular tumors (Leydig cell, interstitial cell) in experimental animals as summarized in Table 6.

Tomatis (1977) reviewed the appropriateness of the subcutaneous (s.c.) injection route as a carcinogenesis bioassay by comparing s.c. with other routes of administration. He surveyed a number of chemicals tested by the s.c. route in rodents to see if there was a correlation between their capacities to induce local and/or distant tumors in one species and their capacities to induce tumors by another route in another species. A total of 102 chemicals, which have been reviewed by the International Agency for Research on Cancer (IARC) and have been tested by the s.c. route as well as by other routes of administration, were surveyed. Of those, 69 were positive for carcinogenic activity when administered by s.c. injection and by another route, and 18 were negative or inconclusive whether given by s.c. injection or another route. Nine were positive only when administered by s.c. injection, and six were negative by s.c. injection and positive by another route. The author concludes that "administration of a chemical by the s.c. route produced what one could call false negative results for six (5.6%) of the 102 chemicals

TABLE 6. ANIMAL TUMORIGENESIS INDUCED BY CADMIUM INJECTION

Author	Species	Compound	Route	Tumor and Incidence
Haddow et al. (1961)	Rats	ferritin containing Cd	s.c.	Sarcomas 8/20 Interstitial cell tumors 10/20
	Mice			Sarcomas 0/20
Heath (1962)	Hooded Rats	Cd powder 0.28 g in 0.4 ml fowl serum	i.m.	Sarcomas 2/10 (later in the study 10 more rats developed tumors; which test group they were in is not stated)
		0.014 g in 0.4 ml fowl serum		Sarcomas 3/10
Heath and Daniel (1964)	Hooded Rats	Cd powder 0.014 g in 0.4 ml fowl serum 0.028 g in 0.4 ml fowl serum	i.m.	Sarcomas 9/10 Sarcomas 6/8 (2 were killed early)
Kazantzis (1963)	Chester-Beatty Rats	25 mg CdS in 0.25 ml physiological saline	s.c.	Sarcomas 6/10
Kazantzis and Hanbury (1966)	Wistar Rats	25 mg CdS in 25 ml physiological saline	s.c. i.m.	Sarcomas 6/10, 6/26 Sarcomas 5/14
		25 mg CdO in 0.25 mg physiological saline	s.c.	Sarcomas 8/10
		0.25 ml physiological saline alone		Sarcomas 0/10
Haddow et al. (1964)	Rats	0.5 mg $CdSO_4.H_2O$ in 1.0 ml sterile distilled water once weekly for 10 weeks	s.c.	Sarcomas 14/20 control 0/15
	Mice	0.05 mg $CdSO_4.H_2O$ in 0.2 ml H_2O once weekly for 11 weeks		0/20 injection site tumors control 0/15

(continued on the following page)

TABLE 6. (continued)

Author	Species	Compound	Route	Tumor and Incidence
Roe et al. (1964)	Rats	0.5 mg $CdSO_4 . H_2O$ in 1.0 ml H_2O once weekly for 10 weeks	s.c.	Interstitial cell tumors 11/15 control 0/15
		0.05 mg $CdSO_4 . 4H_2O$ in 0.2 ml H_2O		Interstitial cell tumors 0/16
Gunn et al. (1963a)	Albino Mice	0.03 mM/kg $CdCl_2$	s.c.	Interstitial cell tumors 20/26 control 0/25
	Wistar Rats	0.03 mM/kg $CdCl_2$	s.c.	Interstitial cell tumors 17/25 control 0/25
Gunn et al. (1964)	Wistar Rats	0.03 mM/kg $CdCl_2$	s.c.	Sarcomas 9/22; control 0/18 Interstitial cell tumors 21/24 control 0/18
Gunn et al. (1967)	Wistar Rats	1.8 mg $CdCl_2$	simultaneous s.c. and i.m.	Sarcomas 10/23 Sarcomas 3/26
Knorre (1970)	Wistar Rats	0.003 mM $CdCl_2$/100 g b.w.	single s.c.	Sarcomas 6/45
Knorre (1971)	Wistar Rats	0.003 mM $CdCl_2$/100 g b.w.	single s.c.	Interstitial cell tumors 10/25
Lucis et al. (1972)	Wistar Rats	0.02-0.03 mM/kg $CdCl_2$ in isotonic NaCl solution	single s.c.	Interstitial cell tumors 13/15 Sarcomas 2/15 (two animals died early)
Reddy et al. (1973)	Fischer 344 Rats	0.03 mM/kg $CdCl_2$	single s.c.	Interstitial cell tumors 16/20 control 0/10
Furst and Cassetta (1972)	Fischer 344 Rats	5 mg Cd powder (suspended in 0.2 ml) synthetic trioctanoin)	2 monthly i.m. injections	Sarcomas 26/50
Favino et al. (1968)	Sprague-Dawley Rats	1 mg/100 g $CdCl_2$	single s.c.	Interstitial cell tumors 6/6

(continued on the following page)

TABLE 6. (continued)

Author	Species	Compound	Route	Tumor and Incidence
Malcolm (1972)	Rats	$CdCl_2$	s.c.	Sarcomas (?) Interstitial cell tumors (?) Experiment not completed at time of publication
Levy et al. (1973)	C.B. Hooded Rats	0.2 mg $3CdSO_4.H_2O$ in 0.2 ml H_2O	weekly s.c. injection into alternate flanks for 2 yrs	Sarcomas 4/25 Interstitial cell tumors 17/25 1 lung adenoma
		0.1 mg $3CdSO_4.8H_2O$ in 0.2 ml H_2O		Sarcomas 1/25 Interstitial cell tumors 17/25 1 malignant lymphoma
		0.05 mg $3CdSO_4.8H_2O$ in 0.2 ml H_2O		Sarcomas 1/25 Interstitial cell tumors 16/25 1 adenocarcinoma of pancreas
		control - 0.2 mg H_2O only		Sarcomas 0/75 Interstitial cell tumors 48/75 1 squamous carcinoma of tongue 1 benign liver cell tumor

tested and, if we accept all the criticisms of this route of administration, false positive results for nine (8.7%) of the 102 chemicals tested. Even so, according to the author, it appears that the s.c. route of administration is not too much worse than any other route of administration.

Inhalation Study in Rats

Takenaka et al. (1982)--

A carcinogenicity study of cadmium administered to male Wistar rats by inhalation has been reported by Takenaka et al. (1982). The animals were placed in a 225 l inhalation chamber for exposure to cadmium chloride ($CdCl_2$) aerosol. Aerosol was generated by atomizing a solution of $CdCl_2$, and airflow through the atomizer was 0.7 l/min. Analytical measurements of cadmium levels were made by collecting aerosol samples in membrane filters in the intake and exhaust of the inhalation chamber. Data in Table 7 show that measured and nominal cadmium levels were quite close. An aerosol centrifuge was used to estimate particle size distribution. Aerodynamic mass median diameters were 0.55 um with an arithmetic standard deviation of 0.48 um.

The animals were initially 6 weeks old and weighed 133 to 135 g. For 18 months, 40 rats per group were continuously exposed to cadmium concentrations of 12.5 ug/m^3, 25 ug/m^3, and 50 ug/m^3. A control group of 41 rats received filtered air during the same period. Following the treatment period, the animals were allowed to survive for an additional 13 months until sacrifice. Body weights were recorded every 3 months during the entire study period. Decedents and survivors were necropsied, and tissues and organs were removed for histopathologic examination. Skulls were decalcified for pathologic evaluation. Samples of liver, lung, and kidney were digested in acid for estimation of cadmium content by atomic absorption spectroscopy.

TABLE 7. NOMINAL AND MEASURED CADMIUM CONCENTRATIONS OF $CdCl_2$-AEROSOLS USED FOR INHALATION
(Takenaka et al. 1982)

Nominal concentrations	ug/m^3	50.0	25.0	12.5
Measured concentrations	ug/m^3	50.8	25.7	13.4
Standard deviation	ug/m^3	5.9	3.6	2.1
Number of measurements	---	212	220	210

Differences in body weights (Table 8) and mean survival times (Table 9) among control and treated animals were not statistically significant ($P > 0.05$).

A dose-related increase in the incidence of primary lung carcinomas in treated animals was evident as shown in Table 9. The first epidermoid carcinoma and the first adenocarcinoma were found 20 and 22 months, respectively, after treatment commenced. Several treated rats also developed adenomas and nodular hyperplasia in the lung. Metastases to the regional lymph nodes and the kidneys and invasion into the regional lymph nodes and the heart occurred in some rats with lung carcinomas. No lung tumors were found in control animals.

Nonneoplastic lesions and various tumors in other organs were found in both control and treated animals. None of these additional tumor types and nonneoplastic lesions was significantly ($P > 0.05$) different among the four groups.

The data in Table 10 show that cadmium was retained in lung, liver, and kidney of survivors for as long as 13 months after cessation of exposure. Anaylsis of these tissues indicates that cadmium was absorbed and circulated throughout the body and that, although the lung was the target organ for carcinogenicity, the kidney retained the largest amount of cadmium. Increases in cadmium levels were dose-related in liver in all treatment groups and in lung and kidney in the mid-dose and high-dose groups. Pathologic changes apparently were not observed in kidney and liver, thus suggesting that the cadmium levels found did not have a toxic effect in these tissues.

The authors attributed their success in demonstrating cadmium carcinogenicity to: 1) performance of a long-term study using $CdCl_2$ aerosols that were retained at a rather high level in the lungs after

TABLE 8. AVERAGE BODY WEIGHTS OF RATS EXPOSED TO CADMIUM CHLORIDE
(Takenaka et al. 1982)

Exposure groups	Average Body Weights (Months After the Beginning of the Inhalation)					
	0	3	7	10	12	15
Control	135.2* (4.8)	333.3 (27.4)	385.2 (30.5)	411.6 (31.2)	422.9 (31.7)	425.1 (31.8)
12.5 ug/m^3	135.1 (6.6)	320.9 (29.4)	375.3 (37.1)	405.2 (39.4)	417.2 (41.4)	420.0 (38.8)
25 ug/m^3	133.4 (6.7)	326.6 (28.6)	382.1 (32.1)	410.0 (32.8)	425.7 (35.9)	428.3 (36.1)
50 ug/m^3	133.3 (6.7)	323.5 (29.0)	375.1 (32.2)	403.2 (34.8)	417.0 (36.6)	422.0 (38.5)
	18†	21	24	27	30	
Control	434.9 (32.4)	428.2 (31.4)	406.2 (41.3)	405.7 (31.3)	367.3 (39.8)	
12.5 ug/m^3	424.6 (41.0)	421.8 (41.2)	409.5 (45.9)	408.4 (40.9)	372.5 (41.8)	
25 ug/m^3	437.6 (38.1)	441.2 (37.7)	429.2 (45.9)	423.9 (37.6)	375.4 (47.8)	
50 ug/m^3	424.3 (40.6)	424.9 (43.8)	415.2 (42.6)	398.4 (35.8)	357.8 (41.5)	

*Mean value (± S.D.).
†End of the inhalation.

TABLE 9. SURVIVAL TIMES AND LUNG CHANGES OF WISTAR RATS AFTER THE EXPOSURE TO $CdCl_2$ AEROSOLS (Takenaka et al. 1982)

				No. of Rats With Lung						
Exposure Groups	Initial No. of Rats	Survival Time in Weeks Mean Value $\pm$ S. D.	No. of Rats Examined Histologically	Adenomatous proliferation	Adenomas	Carcinomas				
						adeno	epidermoid	combined epidermoid and adeno	mucoepidermoid	Total (%)
Control	41	122 ± 19	38*	1	0	0	0	0	0	0
12.5 ug/m^3	40	119 ± 17	39†	6	1	4	2	0	0	6(15.4%)#
25 ug/m^3	40	125 ± 15	38§	5	0	15**	4	1	0	20(52.6%)**
50 ug/m^3	40	116 ± 23	35¶	3	1	14**	7	1	3	25(71.4%)**

*Two rats died during the first 18 months; another rat was not examined because of autolysis.
†One rat was not examined because of autolysis.
§Two rats were not examined because of autolysis.
¶Three rats died during the first 18 months; two other rats were not examined because of autolysis.
#P = $\leq$ 0.01.
**P $\leq 1.0 \times 10^{-5}$.

TABLE 10. CONCENTRATION OF CADMIUM IN LUNGS, LIVER, AND KIDNEYS OF RATS EXPOSED TO $CdCl_2$ FOR 18 MONTHS (13 MONTHS AFTER THE END OF THE INHALATION) (Takenaka et al. 1982)

Exposure Groups	No. of Rats	Cadmium concentration (ug/g wet weight) in Lungs	Livers	Kidneys
Control	9	0.03	0.1 ± 0.1*	0.3 ± 0.1
12.5 ug/m^3	6	5.6 ± 1.0	2.2 ± 0.6	13.5 ± 3.2
25 ug/m^3	9	4.7 ± 1.5	5.9 ± 1.5	16.4 ± 3.6
50 ug/m^3	9	10.4 ± 4.2	13.5 ± 3.0	33.6 ± 10.7

*Mean value ± S.D.

cessation of exposure, and 2) continuous observation of the animals over an extended duration (31 months). Most of the lung carcinomas were detected after the 27th month of the study.

Intratracheal Studies in Rats

Sanders and Mahaffey (1982)--

Sanders and Mahaffey (1982) evaluated the carcinogenicity of cadmium oxide (CdO) in male Fischer 344 rats. Four groups of 46 to 50 rats each were treated as follows: Group 1 (untreated controls) received one intratracheal instillation of 0.9% sodium chloride solution (the dosing vehicle); Group 2 was given an intratracheal instillation of 25 ug CdO when 70 days old; Group 3 received intratracheal instillation of 25 ug CdO when 70 and 100 days old for a total dose of 50 ug; Group 4 was given intratracheal instillations of 25 ug CdO when 70, 100, and 130 days old for a total dose of 75 ug. The authors stated that the 25 ug dose was 75% of the LD_{50} by the route of administration used. Instilled CdO had a count median diameter of 0.5 um.

The animals were allowed to survive until spontaneous death. All animals were necropsied, organs were weighed, and tumors, lesions, and major tissues and organs from all rats except 12 lost due to autolysis or cannibalism were examined histopathologically.

Median survival times were 793, 824, 785, and 788 days for Groups 1, 2, 3, and 4, respectively. Survival times and organ weights (body weights were not obtained) were similar ($P > 0.05$) between control and treated groups. Statistical analysis of tumor data by life table and contingency table methods revealed no significant ($P > 0.05$) differences among the four groups. Lung tumor findings consisted of adenocarcinomas in two rats in Group 3.

Cadmium, as CdO, was not carcinogenic under the conditions of this study; however, the protocol used may not be as sensitive an indicator of the carcinogenic potential of cadmium as a design with lifetime exposures by inhalation, particularly in reference to the carcinogenicity study by Takenaka et al. (1982) discussed herein. Lung tissue was not analyzed for cadmium content in the Sanders and Mahaffey (1982) study. However, clearance of 80% of an intratracheally instilled dose of 15 ug ^{109}CdO from the lung in male Fisher 344 rats, with an elimination half-life of 4 hours and an experimental duration of 2 weeks, has been observed (Hadley et al. 1980) as well as repair of lung tissue to Type 1 alveolar epithelium by 2 weeks following an intratracheal instillation of 25 ug CdO into male Fischer 344 rats (Hadley et al. 1980). Hence, a lifetime inhalation exposure to CdO also might have presented a stronger challenge for carcinogenicity by providing a greater cumulative dose of cadmium within target (lung) tissue.

Drinking Water Studies in Rats and Mice

Schroeder et al. (1964, 1965)

Schroeder et al. (1964) conducted two lifetime exposure studies (survival up to 21 months) in Swiss mice. The animals were given drinking water containing 5 ppm of cadmium acetate. The exposure level was purposely low to simulate the human experience, according to the authors. Only males experienced decreased longevity in comparison with the controls. The exposed males had fewer "visible" tumors (1/>50) than the controls (11/>50), possibly related to shortened lifespan ($P < 0.005$).

In another lifetime exposure study by Schroeder et al. (1965), male and female Long-Evans rats ingested 5 ppm cadmium acetate in water as the sole source of fluid; the treated group developed 28/84 tumors versus 24/70 in controls. The authors stated that "no significant differences appeared among the various groups as to type of tumor." This study, like the 1964 study, was complicated by being performed in a low metal environment with a diet low in many trace metals. When the essential trace element Cr(III) was added to the diet of one group of rats devoid of cadmium, they thrived better than the control group and had 34/71 tumors (Schroeder et al. 1965).

Malcolm (1972)--

Malcolm (1972) gave male Chester-Beatty hooded rats up to 0.2 mg of cadmium sulphate subcutaneously and up to 0.8 mg weekly by stomach tube for 2 years. In another experiment, Swiss mice were given doses of cadmium sulfate in distilled water up to 0.02 mg/5g of body weight subcutaneously at weekly intervals for 2 years. Except for a few sarcomas seen in the rats given subcutaneous injections and Leydig cell tumors (both also seen in the controls), these studies were negative at the time reported.

Levy and Clack (1975)--

Experiments with male specified pathogen-free Chester-Beatty hooded rats, using doses of 0.087, 0.18, and 0.35 mg/kg of cadmium sulphate in distilled water given by gastric instillation once weekly for 2 years, were carried out by Levy and Clack (1975) with no difference in tumor incidence in exposed and control groups. It is noted, however, that this particular strain of rats has a very high lifetime incidence of spontaneous interstitial cell tumor formation (75% in the untreated control group), such that "if exposure to Cd had any effect on the incidence of the lesions it was entirely overshadowed by their spontaneous occurrence," according to the authors. Effects on the prostate were especially scrutinized, with no neoplastic lesions observed.

Dietary Studies in Rats

Loser (1980)--

A 2-year oral carcinogenicity study of Wistar rats given cadmium chloride ($CdCl_2$) was carried out by Loser (1980). Doses of 1, 3, 10, and 50 ppm were given in food to 50 male and 50 female rats, with 100 controls of each sex. Food consumption was similar in all the test groups. The mean body weights of treated males were significantly reduced ($P < 0.01$) at the highest dose level. Other than reduced weight in the high-dose males, the male and female treatment and control groups were comparable for weight, mortality, and tumor incidence.

U.S. Food and Drug Administration (1977)--

An unpublished chronic toxicity study of $CdCl_2$ was conducted at the U.S. Food and Drug Administration (U.S. FDA 1977). The compilation of animals examined pathologically shows that six groups of Charles River COBS (SD) rats, each consisting of 26 to 32 males and 26 to 29 females, were studied. These groups were given 0 (untreated controls), 0.6, 6, 30, 60, or 90 ppm $CdCl_2$ in the diet for 103 weeks. Five males and five females per group were sacrificed at 24 and 52 weeks. All animals were necropsied, and tissues, organs, and tissue masses were examined histopathologically. Kidney tissue from five or fewer males in each sacrificed group was evaluated by electron microscopy; sections of liver and kidney from these animals were stained to assess fibrosis, lipid content, liver glycogen, and the basement membrane of tubuli and Bowman's capsules in kidney.

No significant ($P > 0.05$) differences in survival between control and treated groups were reported, and, excluding interim sacrificed animals, no more than two animals per group died before 77 weeks. Results of necropsy and histopathologic and histochemical evaluations did not show treatment-related effects. Electron microscopy revealed dose-related changes as small cytoplasmic lipid droplets in renal tubular epithelium, increased numbers of residual bodies in renal nephron cells, and swelling and sloughing of cells in distal tubular epithelium and the collecting ducts of the kidney.

Cadmium, as $CdCl_2$, was not carcinogenic in this study, but a stronger evaluation of cadmium carcinogenicity possibly could have been made through the use of larger group sizes and, as suggested by the lack of overt toxicity in treated groups, higher doses.

Cadmium appears to be much less potent by ingestion than by inhalation in terms of the overall carcinogenicity regardless of the site of cancer induction. For example, the total dose of inhaled cadmium in the Takenaka et al (1982) study, where the rats developed a 71% incidence of lung cancer, was about 7 mg (0.25 m^3/day x 0.05 mg/m^3 x 365 days/year x 1.5 years). By contrast, in the Schroeder et al. (1965) drinking water study in rats, which had one of the smallest total doses of all the ingestion studies, a total dose of about 60 mg (5 ppm x 0.05 x 0.35 kg x 730 days) induced no cancer response. If we assume a 10% upper-limit of detection of tumors in the Schroeder et al. (1965) study, the highest reasonable cadmium potency via ingestion is about 0.0017 (0.1/60), campared with a potency of about 0.1 (0.7/7) for inhalation.

While it is possible that cadmium is not carcinogenic by ingestion at all, the negative animal evidence can only set an upper-limit on the carcinogenic potency of ingested cadmium, which in the rat appears to be about two orders of magnitude less than for inhalation.

Summary

Evidence for the carcinogenicity of cadmium and certain cadmium salts in experimental animals has been obtained from studies of cadmium and cadmium salts subcutaneously administered into mice and rats, as summarized in Table 9, and the inhalation exposure study of cadmium chloride aerosol in rats by Takenaka et al. (1982). Oral-intake and inhalation studies were termed inadequate by the IARC in 1976, apparently on the basis of the relatively small doses employed and the small percentage of absorption from the gastro intestinal tract; however, the studies by Takenaka et al. (1982), Sanders and Mahaffey (1982), and Loser (1980) were not reviewed by the IARC. Schroeder's work was specifically designed to simulate human exposure, and, for the most part, the doses given seem realistic for this purpose; however, the doses used were apparently below the maximum tolerable doses usually used today in attempting to establish the carcinogenic potential of various substances.

EPIDEMIOLOGIC STUDIES

Epidemiologic information dealing with the relationship between cadmium exposure and prostate cancer has been obtained from mortality studies. A major difficulty with mortality studies involves survivorship. As survivorship has been improving since the 1940s through the present time, detecting an increased prostate cancer incidence in any group based on mortality from this disease has become increasingly difficult; prostate cancer victims are living longer and, more often than in the past, dying from other causes (Robbins and Angell 1976).

One of the lowest death rates for this site is in Japan where the age-adjusted rate in 1974-75 was 2.4/100,000, although Japan has the highest per capita intake of cadmium in the world. On the other hand, Sweden has the highest age-adjusted death rate from prostate cancer (21.9/100,000 in 1974-75) in the world, but a low daily intake and low body burdens of cadmium (Kjellstrom et al. 1978). Per capita rates, of course, include many people who are not exposed to the agent in question.

There are 12 epidemiologic studies reviewed here that deal specifically with cancer risks resulting from cadmium exposure. Although five of these were reviewed in the OHEA Health Assessment Document for Cadmium (May 1981), they are covered here also for the convenience of the reader.

Potts (1965)

Potts (1965) reported the results of a clinical study of an unstated number of current and former employees of a British alkaline battery factory who were exposed to cadmium oxide dust beginning in 1920 and ending in 1963. In 1946 the manufacture of these batteries was moved to a new location not far from the site of the earlier factory. The first measurements of cadmium dust in the air were made in 1949. At this time, cadmium in the air varied from 0.6 to 2.8 mg/m^3 of air in the platemaking and assembly shops and 236 mg/m^3 in the negative active material department. After the installation of local exhaust ventilation in 1950, cadmium in the air was reduced to less than 0.5 mg/m^3. Improvements to the exhaust system in 1956 further reduced the dust to less than 0.1 mg/m^3. The policy at the time of the study's publication was to take steps to reduce the exposure whenever the measurement of cadmium dust exceeded 0.5 mg/m^3.

Of 70 battery workers for which Potts's clinic had medical records and who were exposed for at least 10 years, proteinuria was observed in 44%. Although no comparison group was provided, this number is probably excessive since proteinuria is the result of renal tubular dysfunction. A 200-248 ug/day cadmium dietary intake over a 50-year exposure period is required to produce a critical renal cortex concentration that is associated with renal dysfunction. Only 1% of Americans ingest more than 50 ug/day (U.S. EPA 1981). However, the author did note that the earlier studies of the urine protein of cadmium-exposed workers in this same plant revealed "similar characteristics" as those of the present study. Four individuals with persistent proteinuria were examined further. Two of them ultimately died. Kidney function tests prior to death revealed no abnormalities nor were any gross abnormalities observed following microscopic examination of the kidneys of the deceased.

In a second phase of this study, Potts claimed that a "careful search" produced a total of 74 men who had been exposed to cadmium dust for more than 10 years and eight of them had died. The author did not reveal what was searched, whether it was his clinic's medical records or employment records of the factory, nor did he say how these 74 men relate to the 70 battery workers mentioned earlier. Furthermore, the source of his death information on the eight was not available. Five of the eight deaths were due to cancer and three of these were cancer of the prostate. The death data from Potts's paper is summarized in Table 11. Presumably, the death information on these eight came from Potts' clinical files, although it is not stated. That the author made any attempt to determine the vital status on the remaining 66 is unclear. Since all of the deaths occurred in the early 60s and nearly all had lengthy exposures, this implies that they were all at risk to the highest cadmium dust levels during their earlier years of employment prior to 1950. No information is given on workers exposed for fewer than 10 years.

In the absence of selection bias (a distinct possibility if clinical records were used), the distribution of the eight deaths is striking as was noted by the author. But because of the possibility of selection bias, lack of a comparison group, and an unknown age structure of the population of 74, it is impossible to say if the observation of three prostate cancer deaths is statistically significant. Therefore, this study provides only the suggestion of an association of prostate cancer and exposure to cadmium.

Kipling and Waterhouse (1967)

Kipling and Waterhouse (1967), in a letter to the Lancet, reported on 246 workers who had been exposed for a minimum of one year to cadmium oxide dust. The authors compared the number of cancers observed from several sites with the number expected from those sites based on incidence rates from the Birmingham Regional Cancer Registry. The number of observed cancer deaths of the prostate was significantly greater than expected (4 observed vs. 0.58 expected, $P < 0.003$). Three of the four prostate cancer cases are the same as those reported in Pott's paper (personal communication from Kipling to the IARC in 1976) indicating that there is some acknowledged overlapping, and

TABLE 11. MORTALITY DATA FOR CADMIUM WORKERS EXPOSED FOR MORE THAN 10 YEARS (Potts 1965)

Year of Death	Age	Length of Cadmium Exposure (yrs)	Cause of Death
1960	65	31	Auricular Fibrillation
1960	75	14	Carcinoma of Prostate
1961	65	37	Carcinoma of Prostate
1962	63	34	Bronchitis and Atheroma
1962	78	18	Bronchitis
1963	53	35	Carcinoma of Bronchus
1964	65	38	Carcinoma of Prostate
1964	59	24	Carcinomatosis

therefore they cannot be said to be independent studies. No significant difference between observed and expected was found for cancer of the bronchus, bladder, testis, or for cancers of all sites.

Latency period, although obliquely referred to in the letter, is poorly addressed. Furthermore, the letter states that expected cases were calculated by "computing the number of cases of cancer which would be expected to occur in such a group of men of known age" and by excluding the time spent in other jobs or retirement. It is not clear how the latter was to be done; the discussion is sketchy at best. The authors mention that "judging from work in similar fields, fairly short exposure may be sufficient to initiate a tumor." Whether this generalized conclusion by the authors can be extended to the specific case of cadmium exposure and cancer remains uncertain. Failure to allow for a sufficient latency period weakens the significance of the findings. Because of these problems and the lack of an adequate discussion of the derivation of expected deaths, the results, although statistically significant, cannot be considered definitive with respect to the carcinogenicity of cadmium.

Humperdinck (1968)

Humperdinck (1968) reported on mortality among 536 people who worked or had worked at an alkaline dry cell battery plant during the period 1949-67 and who had been exposed to cadmium hydroxide and "to a large extent nickel hydroxide." Seventeen of the 536 had died, five from cancer. Of the five who died from cancer, two died from lung cancer, one from liver cancer, one from prostate cancer, and one from cardiac cancer. The length of exposure to cadmium for these cases was: lung, 2.3 years and 9.3 years; liver, 3.5 years; prostate, 6.4 years; and cardiac, 3.0 years.

There was no comparison group for the 1949-67 time period. The author did compare, however, the average of the cancer death rates for the years 1963-66 of the city where the plant is located with the average 1963-66 rate of the whole plant and the average 1963-66 rate for the departments of the plant where there was exposure to cadmium hydroxide. The author did not state whether these rates were age-adjusted, race-adjusted, or sex-adjusted. No difference among the three rates was found. No difference in the proportion of lung cancer deaths between the city population and the plant population was found either. The proportion of lung cancer deaths for the department where cadmium exposure occurred was not reported.

Previously, Baader (1951) had reported on "20 to 30 males and females" suffering from chronic cadmium poisoning at the same dry cell plant. Of this group, Humperdinck reported that four of eight had died, one of lung cancer; these four are included in the seventeen deaths described previously. No mention is made of any of the other "20 or 30" workers.

Because Humperdinck found no excess cancer mortality amoung workers exposed to cadmium when compared to the city population or to the plant population as a whole, he concluded that there was insufficient information to establish an association between cadmium and cancer.

A major weakness of this study is that it did not include an appropriate comparison group for the years of the study, 1949-67. Comparison of average death rates for the years 1963-66 among the city, plant, and cadmium departments is not appropriate since it is not known whether all workers in the cadmium departments in 1963-66 had experienced a latency period of sufficient duration to have developed cancer. Second, there is no indication that the city population or the population of the rest of the battery plant is similar to the cadmium-exposed group in terms of race, sex, smoking habits, age, etc. to make an objective comparison. Third, had a proper comparison group been used and an increase in cancer among workers exposed to cadmium been demonstrated, a possible confounding variable would have been the nickel exposure, since nickel has previously been associated with cancer of the lung, nasal sinus, large intestine, mouth, and pharynx (Fraumeni 1975).

In conclusion, the design and methods of Humperdinck are inadequate to assess whether an association between cadmium exposure and cancer exists for the workers in his study.

Holden (1969)

Holden (1969), in a letter to the Lancet, reported on 42 men exposed to cadmium fumes from 2 to 40 years. He stated that six of the men had been exposed to concentrations of cadmium in excess of 4 mg/m^3 and the remainder had been exposed to an average concentration of 0.1 mg/m^3. The author reported that there was one case of carcinoma of the prostate and one case of carcinoma of the bronchus.

No evaluation of the cancer risk of cadmium can be made from this article since the author did not report important variables such as the age, time since first exposure, and smoking history of each worker.

Kolonel (1976)

Kolonel (1976) compared the cadmium exposure of 64 cases of renal cancer to 197 nonmalignant digestive disease controls and 72 colon cancer controls. According to the author, "a cancer control group was included to address the problem of potential noncomparability" between cases and controls when a noncancer control group is used. Cases and controls were taken from patients admitted from 1957 to 1964 to Roswell Park Memorial Institute, Buffalo, New

York. Cadmium exposure was assessed using data on occupational exposure, cigarette smoking, and dietary intake. A person was considered to have experienced occupational exposure to cadmium only if he had worked for one or more years at a high risk job within a high risk industry. High risk industries included electroplating, alloy-making, welding, and the manufacture of storage batteries. A person was considered to be exposed to cadmium through smoking if he had at least 10 "pack-years" of cigarette use during a lifetime. Dietary exposure to cadmium was determined by applying reports of cadmium content in foods to individual dietary histories based on a frequency recall for a one-week period. An individual was considered exposed through diet if his mean daily intake exceeded the third quartile, determined from the distribution of intakes for the noncancer control group.

The author found that the odds[1] of developing renal cancer in occupationally-exposed patients who smoked was 4.4 when compared to controls who also smoked and had nonmalignant diseases of the digestive system. This is significant at $P < 0.05$. The odds of developing renal cancer in patients who were occupationally-exposed was 2.5 ($P < 0.05$) when compared to colon cancer controls. The latter is not significant ($0.05 < P < 0.10$). Because of the finding of a greatly increased risk[2] when the effects from smoking and occupational exposure are added together, the author concluded that the effects of smoking and occupational exposure must be synergistic.

The odds of developing renal cancer when consideration is given to cadmium exposure through cigarette smoking only, and separately through diet only

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[1] Although the author referred to relative risk in his article, it is more correct to use the term odds ratio or estimated relative risk.

[2] Risk in this context is an estimated relative risk derived by use of the odds ratio.

(utilizing colon cancer controls), were 1.2 and 1.6, respectively, neither of which were significant ($0.05 < P < 0.10$, two-tailed).

A major criticism of this study is the confounding exposures to other industrial materials in the electroplating, alloy-making, welding, and manufacture of storage batteries industries. The author had stated that renal cancer, as a result of cadmium exposure, is biologically plausible because, of all the organs, the kidney concentrates cadmium most. Furthermore, Kolonel pointed out, from an earlier study by Ellman (1959), that the kidney contains the body's highest concentration of sulfhydryl groups which are often found in zinc-containing enzymes. Cadmium inhibition of a variety of sulfhydryl-containing enzymes has been reported, the author notes, and this may be the mechanism of action. The kidney concentrates many trace metals, however, and a variety of metals are found in the industries mentioned above, including nickel, lead, and zinc. Also, it is interesting to note that the odds ratio for occupational exposure to cadmium is significant ($P < 0.05$) only when compared to noncancer controls, but not significant ($0.5 < P < 0.10$) when compared to colon cancer controls. This indicates that the renal cancer cases may not be comparable to the noncancer cases, and selection bias may have occurred.

Smoking has previously been associated with kidney cancer (Wynder et al. 1974, Schmauz and Cole 1974, Kahn 1966, Hirayama 1977) as well as cancers of other sites. Although cadmium may be the carcinogen in the tobacco smoke which causes kidney cancer, the issue is confounded by the presence of many other carcinogens in the smoke. Although the smoke may serve only as a possible synergist or as a carrier mechanism for cadmium exposure from other sources, it remains to be demonstrated, as indicated above, that cadmium is the agent of concern in smoking.

In conclusion, Kolonel's study provides suggestive, but not sufficient evidence that cadmium is a renal carcinogen. More studies, epidemiologic and animal, are necessary to adequately address the issue.

Lemen et al. (1976)

Lemen et al. (1976) conducted an historic prospective study on 292 white male employees of a cadmium smelter who had worked a minimum of 2 years in the smelter sometime during the period from January 1, 1940 to December 31, 1969. Vital status was determined for this group through January 1, 1974. Death certificates listing the cause of death were acquired on 89 of a reported 92 deceased. Some 20 (6.8%) remained lost to follow-up. For comparison, expected deaths by cause were generated through a modified life table technique based upon the product of person-years times the corresponding age, calendar time, and cause-specific mortality rates for the total United States white male population.

The authors stated that the smelter was engaged in the production of cadmium metal and cadmium compounds. However, they reported that some lead was also produced. The plant ceased full-scale lead production in 1918 and began to produce arsenic instead. In 1925, arsenic production ceased with the start of cadmium production at that time. The authors cited a previous industrial hygiene survey in 1947 that reported average air concentrations of cadmium fumes ranging from 0.04 to 6.59 mg/m^3 and cadmium dust at 17.23 mg/m^3. The authors state that most operations had concentrations lower than 1.5 mg/m^3. The present study included a 1973 industrial hygiene evaluation of cadmium dust levels which stated that 8-hour time-weighted average (TWA) gross concentrations of cadmium ranged infrequently up to 24 mg/m^3, but generally remained below 1 mg/m^3. The authors reported that in 1973 a respirator program was in use at the plant which allegedly reduced exposure by a factor of 10. However, respirators have not usually been found to be useful in other settings as workers tend to remove them because of their inconvenience. Two air measurements taken in the preweld department showed that besides air concentrations of 74.8 and 90.3 mg/m^3 of cadmium, arsenic was measured at 0.3 and 1.1 ug/m^3. This is about 1% of the cadmium measurement. In the retort department, however, where cadmium concentration measured 1,105 ug/m^3, arsenic measured 1.4 ug/m^3, which was about 1/1,000th that of cadmium. On the other hand, analyses of bulk samples revealed 42.2% to 70% cadmium, 3.53% to 6% zinc, 0% to 4.3% lead, and 0.02% to 0.3% arsenic. The remaining ingredients were not identified. The authors concluded that the exposures from the remaining ingredients were insignificant.

A statistically significant excess of total malignant neoplasms (27 observed vs. 17.6 expected, $P < 0.05$) was found, as was also the category of malignant respiratory disease (12 observed vs. 5.1 expected, $P < 0.05$). Without regard to latent effects, an excess of prostatic cancer was reported by the authors to be not significant (4 observed vs. 1.15 expected). However, utilizing a one-tailed Poisson variable, the CAG found this observation to be statistically significant ($P < 0.05$). After a lapse of 20 years from initial exposure, the finding of a statistically significant excess in prostatic cancer (4 observed vs. 0.88 expected, $P < 0.01$) was even stronger.

Information concerning exposure and latency of the four prostatic cancer cases is given in Table 12.

Of the 12 malignant respiratory cancer cases, the cell types of eight were known. Three were squamous cell carcinoma, one was undifferentiated small cell carcinoma, three were anaplastic, and one was an oat cell carcinoma,

TABLE 12. PROSTATE CANCER DEATHS AMONG CADMIUM SMELTER WORKERS WITH MORE THAN 2 YEARS EXPOSURE
(Lemen et al. 1976)

Case	Age	Exposure	Latency	Date of Death
1	71	4	32	2/26/72
2	77	13	25	3/19/68
3	79	18	31	12/10/60
4	64	17	26	4/3/51

according to the authors. Unfortunately, smoking histories were not available for any members of the cohort. Therefore, the confounding of the results due to smoking cannot be assessed. Only in one other study by Holden (1969) of cadmium-exposed workers does the author take note of a single case of bronchogenic cancer appearing in 42 workers exposed to cadmium on which the author did not comment. Furthermore, Lemen et al. reported the presence of other substances in the smelter either known or suspected of causing cancer. Arsenic, for example, has been shown to be a human lung carcinogen. Thus, any conclusions that are made regarding the carcinogenic potential of cadmium should be tempered with the knowledge that arsenic, lead, and zinc were also known to be present in the atmosphere of the smelter. Other constituents of the processed ore are left unidentified (the percentage content of the ore does not add up to 100%).

However, when consideration is given to the fact that the vital status of 6.8% of the study cohort remains unknown, additional causes of death found in this group of 20 people potentially may add a prostate cancer or two to the observed deaths. In contrast, expected deaths are overestimated by counting person-years to the cut-off date for these same individuals. This could bias downward the finding of an excess risk of prostate cancer and bronchogenic cancer.

This study provides evidence that exposure to cadmium leads to a significant excess risk of prostate cancer. The other metals known to be present have not been shown to be associated with an elevated risk of prostate cancer. On the other hand, the presence of arsenic in the atmosphere of the smelter and the lack of information on smoking habits of the workers casts doubt on the significant association of bronchogenic cancer in these workers with cadmium exposure.

McMichael et al. (1976)

McMichael et al. (1976), as part of an historic prospective study of cancer mortality among rubber workers, followed 18,903 active and retired male workers, aged 40 to 84, for a period of 10 years. They were divided into four separate cohorts each consisting of workers from the four tire manufacturing plants of the companies under study.

The mortality experience during the 10-year observation period was determined from death claims filed with the companies under the group life insurance policy in effect. In three of the four plants, workers were included if they were employed on January 1 1964, whereas in the fourth plant it was January 1, 1963. About 1% were lost to follow-up according to the authors, and death certificates listing cause of death were obtained on 98% of the deceased. Expected deaths were calculated based on the 1968 U.S. male race-age-specific death rates. The calculation of standard mortality ratios (SMRs) utilizing such rates produces an underestimate of the risk. This bias, known as the "healthy worker effect," is a consequence of the selection of the healthiest individuals into a given workforce from the general population from which the expected deaths were derived. Apparently, little turnover occurred in these four plants because the former employees who switched to another place of employment formed the group of 1% lost to follow-up during the 10-year follow-up period.

The total number of deaths equaled 5,106 for an overall SMR of 94. The total number of cancer deaths equaled 1,014 for an SMR of 100, while that for prostate cancer was 119 (103 observed, nonsignificant at $0.05 < P < 0.1$). The author noticed an association of prostate cancer with the compounding and mixing areas of the four plants, work areas that entail contact with metallic oxides (including cadmium oxides). McMichael et al. also noted an association of prostate cancer with three additional work areas (cement mixing, janitoring, and trucking) of one particular plant after "exploratory work-history" analyses were completed for stomach, bladder, and prostate cancer; lymphosarcoma; and Hodgkin's disease at this plant.

The object of this study was not to single out the association of prostate cancer with cadmium exposure as the main topic of study, but rather to examine site-specific cancer mortality, in general, in rubber workers. Hence, the authors found excesses in cancer mortality at a number of different sites but did not test the significance of any. These data (McMichael et al. 1976) are summarized in Table 13. The tests of significance were calculated by the Carcinogen Assessment Group (CAG) using the method of Chiang (1961).

One major problem with this study is that rubber workers are potentially exposed to numerous organic and inorganic chemicals, some of them known or suspected carcinogens, including benzene, which is a known human carcinogen. While cadmium is present in these factories, SMRs may be confounded by exposures other than cadmium. Exposure levels of the many different compounds found in these plants are not given.

A second problem with this study is the relatively short observation time (10 years) from the beginning of the study to its cut-off. This is an insufficient period in which to assess latent effects, and in fact, no data is presented in which latency is considered. This cohort should be followed for several additional years before concluding that there is no effect from cadmium exposure.

While this paper is of interest as a basis for further studies, it is inadequate evidence for associating or not associating cadmium with prostate cancer.

TABLE 13. STANDARD MORTALITY RATIOS (SMRs) BY SITE (McMichael et al. 1976)

Site	Observed Deaths	SMRs	Probability of Occurrence*
Lymphatic leukemia	20	158	0.039
Stomach	80	148	< 0.001
All leukemias	46	130	0.073
Hodgkin's disease	32	129	0.150
Prostate	103	119	0.077
Colon	103	116	0.131
Pancreas	57	103	0.826
Bladder	32	92	0.638
Respiratory	252	85	0.002
Rectum	27	82	0.303
Brain, central nervous system	14	78	0.352
All cancer	1014	100	1.000
All causes	5106	94	< 0.001

*Taken from Chiang (1961).

Kjellstrom et al. (1979)

Kjellstrom et al. (1979) reported on an historic prospective cohort study of 269 male Swedish cadmium nickel battery factory workers and 94 Swedish male cadmium-copper alloy factory workers having more than 5 years exposure since the factories began production. As an internal reference group, 328 alloy factory workers not exposed to cadmium were also studied. They were employed in the alloy factory for at least 5 years but not exposed to cadmium. It has been estimated that the average cadmium levels for one of the two factorys were as follows: exceeded 1 mg Cd/m^3 prior to 1947; 200 ug Cd/m^3 between 1962 and 1974; 50 ug Cd/m^3 in 1974; decreased to below 5 ug Cd/m^3 at the time of the study. At the other factory, concentrations were in the range of 100 to 400 ug/m^3 in the mid-sixties and 50 ug Cd/m^3 in 1971 and after. The battery study population was also exposed to nickel hydroxide dust.

National average age-cause-specific death rates and cancer incidence rates were used to generate expected deaths and expected new cancer cases in the two separate study groups. New cases of cancer were found in the battery factory by matching the names of the 269 workers with those of the Swedish National Cancer Register. This was not done with the alloy factory workers. With respect to mortality in the battery factory, 43 deaths occurred between 1949 and 1975 of which eight were due to cancer. This contrasts with 67 expected total deaths during the same period. No further breakdown is given of the cancer deaths and no expected cancer mortality is given. However, the authors state that there was no increase in "general" cancer mortality. Furthermore, the total number of new cases of cancer equaled 15 during the period 1959 to 1975, while the expected number of new cases equaled 16.4 based in incidence data provided by the Swedish National Cancer Register. A breakdown by site is given in Table 14. Only cancer of the nasopharynx was found to be

TABLE 14. EXPECTED AND OBSERVED NEW CASES OF CANCER BETWEEN 1959-1975 IN THE WHOLE GROUP OF BATTERY FACTORY WORKERS (N = 228)
(Kjellstrom et al. 1979)

	Cancer Cases		
Site	Expected*	Observed	Risk Ratios
Prostate	1.2	2	1.67
Lung	1.35	2	1.48
Kidney	0.87	0	0
Bladder	1.07	1	0.93
Colon-rectum	2.25	5	2.22
Pancreas	0.60	0	0
Nasopharynx	0.20	2	10.0†
Other	9.81	3	0.31
All sites	16.4	15	0.91

*Expected deaths based on Swedish National Cancer Register.
†Statistically significantly greater than 1 ($P < 0.05$).

significantly in excess (2 observed vs. 0.2 expected, $P < 0.05$) possibly due to exposure to nickel dust.

In the alloy factory, only "preliminary" calculations of prostatic cancer mortality were done according to the authors; cause-specific mortality or incidence was not examined in these workers. Among 94 exposed workers, four prostatic cancer deaths were noted versus 2.69 expected ($P = 0.29$). In the reference group of 328 nonexposed workers, four prostatic cancer deaths were noted versus 6.42 expected ($P = 0.23$) (Table 15). A corrected "healthy worker effect" risk ratio was derived by dividing the risk of developing prostatic cancer in the exposed by that of the reference group. This equals 2.4 ($P = 0.087$), which is still nonsignificant.

Although the results of these two studies are nonsignificant with respect to prostatic cancer and basically inconclusive due to the small sizes of the study groups, they do suggest a positive association of prostate cancer with exposure to cadmium.

Two problems with this work are apparent. The first is that terminated employees are apparently not included in any of the study cohorts unless they are terminated by death. The resulting cohorts are healthier than the general population because former employees, who would be expected to carry the greatest burden of potential disease, are not represented. These employees are represented in the general population death rates however. The net result is to overestimate the expected deaths, thus masking a potential risk in battery workers.

Second, in the incidence studies, cancer cases occurring in the 1950s would be missed since the Swedish Cancer Registry didn't begin until 1959, thus leading to an underestimate of new cancer cases.

Another potential source of selection bias involves the exclusion of all

TABLE 15. CUMULATIVE EXPECTED AND OBSERVED NUMBER OF PROSTATIC CANCER DEATHS IN 1940-1975 AMONG ALLOY FACTORY WORKERS
(Kjellstrom et al. 1979)

	Prostatic Cancer Deaths			
	Expected	Observed	Risk Ratios	P value
Exposed group	2.69	4	1.49	0.29
Reference group (N = 328)	6.42	4	0.62	0.23

members with incomplete information in the factory files. However, since there is no reason to assume differential selection of subjects for study through this procedure, it may not be a problem.

Holden (1980)

Holden (1980) reported the results of a preliminary cohort mortality study of workers employed sometime from August 1940 to August 1962 and followed to December 31, 1979, in a British cadmium factory, while iron and brass foundry workers in a second factory served as a control. The cadmium factory was further subdivided into two sections for analysis purposes. One section of the building contained the copper cadmium alloy department. In that department, 347 men worked a minimum of 12 months while another 624 men worked a minimum of 12 months in the remaining part of the factory. These were dubbed "vicinity" workers by the author because they worked within the building but not in the copper cadmium alloy department. Another 537 brass and iron workers were employed in the second British factory for a minimum of 12 months and were reported by the author to be similar to workers of the first factory with respect to their social and physical environment.

Industrial hygiene surveys carried out at this factory in 1953 and 1957 indicated a mean level of 70 ug/m^3 (S.D. = 62 ug/m^3) based on 12-hour sampling data in the cadmium copper alloy department, while a mean level of 6 ug/m^3 (S.D. = 8 ug/m^3) was measured in the vicinity. Vicinity workers were manufacturing arsenical copper, and during refining they were exposed to silver and nickel. The author reports that those workers were exposed to considerably less cadmium than were the cadmium copper alloy workers. Follow-up was over 95% complete on all three subcohorts. Expected deaths were generated based upon death rates for England and Wales in 5-year age intervals.

A statistically significant elevated risk of dying from all causes (observed = 158, SMR = 112) was evident in cadmium copper alloy workers. This was repeated even when malignant neoplasms were excluded (observed = 122, SMR = 113). Mortality from neoplasms was not significant in cadmium copper alloy workers except for leukemia (observed = 3, SMR = 441). The author contends that the excess overall was due to pulmonary disease deaths. On the other hand, a statistically significant elevated risk of cancer in general (observed = 72, SMR = 120) was apparent in "vicinity" workers due chiefly to an excess of cancer of two sites, lung cancer (observed = 36, SMR = 138) and cancer of the prostate (observed = 8, SMR = 267). The author attributes the elevated risk of lung cancer in these workers to the presence of other metals such as arsenic. With respect to prostate cancer, he correctly noted the absence of a dose-effect relationship since five of the eight prostate cancers occurred to individuals with less than 15 years exposure; three were exposed only one year if one assumes that "years of exposure" means years of exposure throughout the entire plant. The author attributes only three of the prostate cancer deaths to cadmium exposure. This last observation is somewhat strong in view of the fact that every prostate cancer case occurred more than 15 years after initial exposure. Latency as a factor was not considered in calculating expected deaths so that the actual risk may be greater.

It should be noted that the workforce of any factory may be rotated many times during its operating life. The fact that cadmium alloy workers, under the author's definition, apparently experienced a lower risk of prostate cancer than did "vicinity" workers may not be so unusual. It is possible that several of the eight cases worked in the copper cadmium alloy department as well as in the remaining part of the plant at some time during their working career.

The observed risk of cancer may actually be greater than calculated because of the presence of the healthy worker effect. The fact that this factor is operating in these cohorts is a consequence of the observation that less than expected mortality was seen in the control group not only in the overall risk of death, all causes, (observed = 95, SMR = 88), but also with respect to the risk of cancer (observed = 21, SMR = 88).

Because of the preliminary nature of the findings of excess lung and prostate cancer in "vicinity" workers and further questions that need to be answered regarding the extent of exposure to cadmium, the findings of an excessive risk of prostate cancer in these workers should be regarding only as strongly suggestive. The finding of an excessive risk of lung cancer due to cadmium exposure must be regarded as questionable because of exposure to other metals that could be regarded as potential lung carcinogens.

Kjellstrom (1982)

Kjellstrom (1982), in an updated historic prospective study of a cadmium nickel-battery factory, reported on mortality patterns in a cohort of 619 male employees (including 269 from an earlier study). During the study period from 1951 to 1980, 103 workers died compared to 126.4 expected based on Swedish Mortality Statistics. The highest SMR was urogenital disease with 4 deaths versus 2.5 expected and nonsignificant. Only 4 prostatic cancer deaths occurred versus 3.1 expected. The workers in this study cohort had a minimum of one year exposure to cadmium. The author notes that, based on preliminary data, prostatic cancer mortality may be "more increased than the mortality due to other causes." This was not statisically significant however. Average historic exposure levels within this plant are depicted in Figure 1.

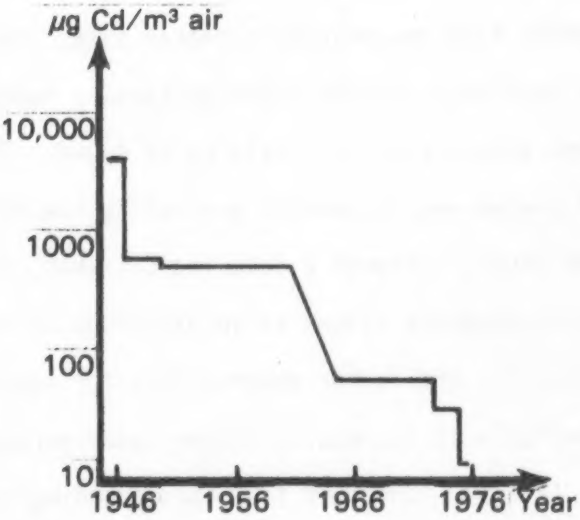


Figure 1. Concentration of cadmium in the air (ug Cd/m^3) from 1949 to 1976. Arithmetic mean of stationary and personal samples. (Kjellstrom 1982).

From 1946 to 1967 there appears to have been a 1,000-fold drop in average exposure levels. A detailed analysis of past and present cadmium exposures in this factory has been published (Adamsson 1979). The author reports that nickel exposure levels have been at least the same as that of cadmium and oftentimes as much as 10 times higher.

Problems with this study are as follows: The records of terminated employees prior to 1945, a group in which the greatest risk is likely to be found, are nonexistent. Almost 31% of this group had less than 2 years duration of exposure to cadmium. Almost 50% of the cohort (301 workers) received their first exposure to cadmium after 1959, which means a large proportion of the cohort had not been followed even 20 years, and thus, there was probably not enough time to evaluate a cancer risk. Furthermore, smoking information was not available for the older workers, a subgroup of the cohort in which the greatest cancer risk is likely to be found. This conceivably could have been the reason why no results evaluating the effects of smoking are presented in the study, although a detailed data-base is reported by the author to be in the development stages as an extension of the study for future follow-ups. Additionally, the author reports that for cancer of the prostate, the rate ratio increases with increasing latency and increasing dose. He reports rate ratios of 1.27, 1.33, and 1.55 corresponding to exposure categories > 0 years, > 1 year, and > 5 years. In the > 1 year exposure duration category, prostate mortality rate ratios of 1.33, 1.44, and 1.81, corresponding to latency periods of 1, 10, and 20 years, respectively, are given. However, since no tabular data is presented, it is not possible to determine how the four observed prostate cancer deaths are distributed into the subcategories alluded to by the author. The author did note that the numbers were too small for the detection of statistically significant differences.

Kjellstrom repeated the above exercise for urogenital diseases. For those with more than 20 years exposure and 20 years latency, 4 observed urogenital deaths occurred versus 0.93 expected ($P < 0.05$). This type of disease was exclusively nephritis of the kidney. Again it is difficult to conclude without evaluation that cadmium exposure was related, although the author himself states that it is "clear that cadmium exposure increases mortality from kidney diseases" after high exposure intensity and long duration of exposure. The author notes a tendency in his data for a slightly increased nonsignificant risk of prostate cancer from exposure to cadmium.

In addition to the main study discussed above, Kjellstrom included discussions of four Japanese studies [Japanese Public Health Association (JPHA) 1979; Shigematsu et al. 1981; Nogawa et al. 1978, 1981] and a description of another planned ecological study by himself and the Department of Epidemiology at the University of Tokyo for which only preliminary findings are available. In this latter ecological study age-standardized death rates in cadmium polluted areas for persons 35 to 84 years of age were compared with the respective rates in non-cadmium polluted areas. Preliminary data, according to Kjellstrom, suggested a nonsignificant tendency toward higher mortality rates in cadmium-exposed areas compared to control areas (an age-adjusted mortality rate of 176 per 1,000 in cadmium-exposed areas versus 139 in the control areas). Prostate cancer and kidney disease mortality rates were also higher in the cadmium-exposed areas, but most of the prostate cancer mortality excess occurred in individuals 85 and over. No significant tests were done. This analysis was reported by Kjellstrom as tending to support the hypothesis of a cadmium effect, but "definite conclusions have to be left until all the analysis are completed."

The first of these (JPHA 1979, Shigematsu et al. 1981) was an analysis of cadmium exposure and mortality in the general environment. According to the author, people in many areas of Japan endure high cadmium exposure up to several micrograms/day from consumption of contaminated rice. In each of four "prefectures" of Japan, age standardized mortality rates were calculated in a cadmium-exposed area and compared to those calculated in a nonexposed reference area of the same prefecture. It was found that cancer mortality rates were generally about the same in the non-polluted areas compared to the polluted areas, but no significance tests were run. Only kidney disease death rates and diabetes death rates were found to be lower in non-cadmium polluted areas compared to cadmium polluted areas. With respect to prostate cancer mortality, two of the polluted areas had higher death rates than did their controls, while in two others the reverse was true according to the author. The author noted that the two prefectures with higher death rates of prostatic cancer compared to their controls are the areas with the "highest likely cadmium exposure to the population." The remaining two prefectures had lower cadmium exposures. The former two prefectures tended to have higher rates of mortality from kidney disease and hyperplasia of the prostate as well. Again, this is an ecological study and thus can only be considered as suggestive of areas for future research.

The second Japanese study (Nogawa et al. 1978) found that in 2,689 men and women over age 50, the village-specific prevalence of low molecular weight proteinuria (LMWP) increased with an increase in the village-specific average cadmium concentration in rice. LMWP was measured by urinary retinol binding protein. Since this ecological study more than likely includes more persons who have never been exposed to cadmium in rice as well as prevalence rates that include persons with prior-existing conditions, possibly introduced long before they were exposed to relatively high concentrations of cadmium in rice, the positive association noted by the author should not be construed to signify a causal association.

Nogawa et al. (1981) conducted a mortality study of the 81 men and 124 women identified in the earlier study as having LMWP. They were followed from 1974 to 1979 as well as the remaining men and women not found to have LMWP. He found a nonsignificant ($P < 0.05$) twofold excess risk of death to men with LMWP and a nonsignificant 1.2-fold excess risk of death to women with LMWP. Mortality rates were based on 27 deaths of males with LMWP and 30 deaths of females with LMWP. A positive association of LMWP with heart disease, cerebrovascular disease, nephritis, and nephrosis was noted. This association raises the spector of a possible confounding effect of hypertension with LMWP. If hypertension is a cause of LMWP, the higher mortality in individuals with LMWP described above may have been a consequence of the hypertension and not necessarily that of LMWP brought on by cadmium exposure as suggested by the author. Thus, the correlation with LMWP may be spurious. Hence, conclusions drawn from this study regarding an association of higher mortality with cadmium exposure must be characterized as certainly no more than suggestive.

Kazantzis and Armstrong (1982 unpublished)

In a recently completed but unpublished cohort mortality study of 6,995 male cadmium workers with at least one year service in one of five British industries (primary producers, copper-cadmium alloy, silver-cadmium alloy, pigments, and plastic stabilizers), the only finding of any statistical significance was that of bronchitis deaths occurring to employees classified as having "high" exposure to cadmium (12 observed versus 2.5 exposed, $P < 0.01$). The excess of bronchitis (chronic respiratory disease) occurred among workers exposed mostly to cadmium fumes, although the author reports a nonstatistically significant excess among those exposed to cadmium dust only; no data is provided to support this finding. Workers who were born after 1940 or who were exposed after 1970 were excluded from the study. The remaining cohort was followed to 1979 when it was reported that 1,902 deaths had occurred versus 2,056 expected (SMR = 93), a shortfall of deaths attributed most likely to the "healthy worker effect." No information is provided concerning the degree of success of the follow-up. Standard mortality rates were presumably based upon British death rates. The entire cohort was divided into three categories, "high," "medium," and "low," according to severity of exposure. Some 3% fell into the high category, 17% were in the medium category, and 80% were in the low category, according to the author. These categories of exposure were devised by industrial hygienists based upon knowledge of past and present processes and working procedures, and upon biological and environmental monitoring results where available. The number of persons who fell into the highly exposed category (certainly no more than 210 persons total) was a rather small number upon which to estimate a risk of prostate cancer.

No other excessive risks of death by cause (including cancer) were present according to the authors. Unfortunately, only one table of tabular results of cause-specific mortality are available in this study by exposure category, and this table has no breakdown by time since onset of exposure. Hence, no consideration of latent effects are given in the study. Therefore, it is impossible to assess the risk of cancer in just that group followed for more than 15 or 20 years after initial exposure to cadmium.

It should be noted that this study is not independent of an earlier study by Sorahan (1981, unpublished). The author acknowledges the inclusion of

3,026 male (43% of the cohort) nickel-cadmium workers who are the subject of a cohort mortality study by Sorahan. (The Sorahan study is critically reviewed following this discussion.) Sorahan also found essentially negative results. Kazantzis himself admits that with respect to both kidney disease and prostatic cancer, the "numbers are too small to exclude an increased risk emerging with time," although he states that if such a risk existed, it "could only be small in terms of extra deaths expected." It is difficult to see how he could make the latter statement since he made no effort to sort out latent effects. This study is, in the opinion of the CAG, inconclusive in terms of finding no risk of prostate cancer or cancer of other sites.

Sorahan (1981)

Sorahan (1981), in a letter to Dr. Roy Albert (dated 2/18/81), related the findings of an historic prospective mortality study of 3,026 nickel-cadmium battery workers employed during the period from 1946 to June 30, 1980, who had worked at least one month. A subset of these same workers was studied earlier by Kipling and Waterhouse (1967), according to the authors. The cohort was derived from workers employed in two separate factories that amalgamated in 1974. The earliest mention of cadmium in the air was reported in 1949. In the platemaking assembly shops, the range was 0.6 to 2.8 mg/m^3, but in the "negative active material" department where cadmium oxide powder was prepared, the levels were reported to be "considerably higher." No numbers are provided. Extensive local exhaust ventilation was installed in 1950, and as a consequence, cadmium in the air was reduced to below 0.5 mg/m^3 in most parts of the factories. By 1967, when a new platemaking department was built, the cadmium oxide dust was reduced to less than the threshold limit value (TLV) of 0.2 mg/m^3. From 1975 on, the plant levels were within the current TLV of 0.05 mg/m^3.

For the purposes of the analysis the author excluded 566 female employees and divided the remaining all male cohort into two subcohorts: 1,066 who were first employed before the amalgamation (1947) and 1,494 who were first employed after the amalgamation.

Standard mortality ratios (SMRs) were computed. Expected deaths were generated assuming that the general population rates were operating on the study cohorts. Overall, the observed numbers of deaths, all causes combined, were slightly less than expected (observed = 591, SMR = 97). With respect to all forms of cancer, there was virtually no difference between observed and expected deaths (observed = 152, SMR = 100). On the other hand, a deficit of cancer deaths occurred to the subcohort of employees employed prior to the amalgamation (observed = 80, SMR = 84). But, in those employed for the first time after the amalgamation, a significantly increased risk of total cancer deaths was apparent (observed = 72, SMR = 129, $P < 0.05$). This increased risk was attributed by the author to a survivor population effect in the latter subcohort. However, in both subcohorts, before 1947 and after 1947, an excessive but nonsignificant risk of cancer of the bronchus was evident (observed = 45, SMR = 114; observed = 32, SMR = 134, respectively). No significant excess risk of prostate cancer occurred in either group (observed = 4, expected = 4.1; observed = 3, expected = 1.9, respectively). Even after consideration was given to the time since first employed, no significant excessive risk was seen in workers 15 years after first employment in any of the following cause of death categories: all causes combined, all cancers combined, cancer of the bronchus, and cancer of the prostate. Nor was there a significant risk of cancer to employees having left employment before completion of one full year of employment and one to 14 years of employment. In an apparant inconsistency, Sorahan reported no prostate cancer deaths in the length of employment category 1 to 14 years; yet in the earlier study by Potts (presumably based upon the same data), three employees whose deaths were attributed to prostate cancer were exposed to cadmium for 14, 37, and 38 years, respectively. Given the cohort definition of Sorahan, at least one of these three cases should be included.

Furthermore, in Sorahan's analysis of latent effects, implicit is the assumption that only terminated employees were considered in the analysis. Presumably, person-years of individuals still employed with the company were not enumerated. Only if the individual left the employment of the company (through death or other cause) would his person-years be counted. This has the effect of reducing the expected deaths by the non-inclusion of person-years of individuals who are at risk of death but are still alive and working. This would tend to bias the SMRs upward.

On the other hand, without regard to latent effects, the study suffers from a type of selection bias known as the "healthy worker effect" brought about by comparison of the observed deaths with expected deaths based upon the mortality rates of England and Wales, thus biasing the result toward the null. Additionally, some 82 persons remain untraced with respect to their vital status. The non-inclusion of the causes of death of the decreased members of this subgroup would tend to bias the SMRs downward.

More seriously, tabular data presented classifies the cohort into two categories of exposure: "exposed" and "non-exposed," although in the "population section" the author described all the jobs in the factories in terms of "high," "slight," and "minimal" exposure to cadmium. It cannot be determined from the text how the three latter categories were reconstituted as "exposed" and "non-exposed" for the purposes of presenting the findings in tabular form. The author's treatment of the subject suggests that some portion of the study population received little or no exposure to cadmium, and if this is so, they should have been excluded from the study group, or else a better definition of intensity of exposure should have been utilized to present the tabular findings. It would have been more appropriate and informative to present the tabular findings in terms of "high, slight, and minimally" exposed groups. Otherwise, the cancer incidence cannot be adequately related to cadmium exposure.

Overall, this paper presented no evidence of an increased risk of prostate cancer in cadmium-exposed workers. However, since many questions remain unanswered concerning the structure of the study, it cannot be said to provide conclusive evidence that cadimum is not carcinogenic.

Summary

Of the 12 epidemiologic studies of cancer in cadmium-exposed people reviewed by the CAG, three (Kipling and Waterhouse 1967, Lemen et al. 1976, Holden 1980) provide evidence of a significant association with prostate cancer. Although the numbers are very small, four cases in each study, a statistically significant positive association was observed ($P < 0.05$) in all three studies.

Three other studies (Potts 1965, McMichael et al. 1976, Kjellstrom 1978) provide the suggestion of a risk of prostate cancer (although statistically nonsignificant) with exposure to cadmium. Potts's clinical study (which provided three of the four prostate cancer cases in the Kipling and Waterhouse study) suffers from a lack of a comparison group. The McMichael et al. study of mortality in rubber workers suggested a positive correlation of exposure to cadmium with prostate cancer. However, the lack of significance even with large numbers (observed = 103, SMR = 119) and the concomitant exposure to other known and suspected carcinogens make this study equivocal. Kjellstrom's "corrected healthy worker effect" risk ratio of 2.4 is still nonsignificant because of small numbers, although it approaches borderline significance at $P < 0.09$, offering the suggestion of a possible association of prostate cancer with cadmium exposure.

Two other studies (Humperdinck 1968, Holden 1969) did not report evidence of an association of prostate cancer with cadmium exposure chiefly because the comparison population was either inadequate to assess a risk (Humperdinck) or absent entirely (Holden). The ninth study (Kolonel 1976) did not evaluate the risk of prostate cancer in cadmium-exposed people but did evaluate the risk of renal carcinoma and cadmium exposure.

An update by Kjellstrom (1982) of his earlier 1979 study again failed to demonstrate a significant risk of cancer of any site. One of the failings of this study was that members of his cohort were not observed long enough to evaluate latent effects. More than half of his cohort received no exposure to cadmium prior to 1959 and thus could not have been followed for even 20 years.

The study by Kazantzis and Armstrong (1982) of 6,994 workers, which included the entire Sorahan cohort, also failed to demonstrate an increased risk of cancer of any site. This study combines cohorts from several different plants each with their own unique exposure history, none of which are necessarily comparable. The main failing of this study, however, is the lack of consideration of latent effects.

Kolonel (1976) found a statistically significant elevated risk of renal cancer in persons occupationally exposed to cadmium, and an even greater risk in occupationally exposed people who smoke, thus raising the possibility of a synergism. The chance of selection bias and concurrent occupational exposures to nickel, lead, zinc, and a variety of metals minimizes the importance of the findings. None of the other human epidemiologic studies reviewed have demonstrated an elevated risk for the development of renal cancer and exposure to cadmium.

Lemen et al. (1976), in addition to a significant risk of prostate cancer, found a statistically significant elevated risk of bronchogenic carcinoma in cadmium smelter workers (12 observed vs. 5.1 expected, $P < 0.05$). But these same workers were simultaneously exposed to low levels of arsenic, lead, and zinc. Arsenic is a known pulmonary carcinogen and thus, confounding cannot be eliminated. Furthermore, smoking habits among workers in the rather small study cohort are not known.

Holden (1980) also noted a statistically significant elevated risk of bronchogenic carcinoma in British cadmium workers who worked in the "vicinity" of the cadmium copper alloy department. However, the author attributed the excess risk to the presence of other metals, such as arsenic.

The Sorahan (1981) study, which reportedly includes the same group of workers evaluated by Kipling and Waterhouse, is negative for an increased risk of prostate cancer. Separation of the study group according to "exposed" versus "unexposed" is unclear. Since the control group constitutes the population of England and Wales, the "healthy work effect" may bias the result downward. Furthermore, although it is not clear whether the first four tables include only terminated employees, the rest of the tables explicitly state that is the case. If only terminated employees were analyzed, the SMRs would be biased upward. For these and other reasons, the study is inconclusive to determine an association between cadmium exposure and cancer.

It might be of interest to estimate the likelihood that positive results in the five independent study populations discussed above could occur by chance alone under the hypothesis that cadmium had no effect on deaths due to prostate cancer. To test this hypothesis, a formal statistical test of the joint information contained in all the studies can be made in the following manner. Often the same hypothesis is tested in a series of independent studies where an estimate of the probability of rejecting a true hypothesis, the "P" value, is calculated for each study.

The results of any one individual study may be only marginally significant, but the series of consistent results among the studies gives the appearance of much stronger evidence than any one study. It would be desirable to combine the results of the studies in some manner to obtain some overall statement about the "P" value of the series of experiments in order to obtain a joint result. However, often due to differences in how the data were analyzed and obtained in the studies, this approach was not possible. Sir R.A. Fisher developed a simple, straightforward method to analyze data in this situation where the only information needed is the "P" value for each of the studies. He showed that the statistic

$$S = -2 \sum_{j=1}^{m} \log_e P_j$$

is distributed as an X^2 value with 2m degrees of freedom, where Pj is the "P" value for the jth study, and m is the total number of studies. However, caution must be taken in utilizing this approach to be sure that all methodologically acceptable studies that have been used to test the required hypothesis are included in the series and that all of these studies are independent of each other. If studies that did not produce a "significant" result are not reported in the literature, or one study uses part or all of the actual data from another study, the proposed use of the Fisher method could lead to a biased result.

In the present situation a total of five independent studies have been conducted testing the hypothesis of no association between exposure to airborne cadmium and prostate cancer. These studies, along with the resulting "P" values, are as follows:

Study	Source of Exposure	"P" Value
Kazantzis and Armstrong (1982)	Combined-Primary Production Cadmium Copper Alloy Cadmium Silver Alloy Plastics Stabilizer Pigments	0.52
Lemen et al. (1976)	Cadmium Smelter	0.013
McMichael et al. (1976)	Rubber Plants	0.077
Kjellstrom et al. (1978)	Cadmium Nickel Battery Plant Cadmium Copper Alloy Plant	0.375
Holden (1980)	Cadmium-Copper Alloy	0.010

Applying Fisher's method, we find that the statistic

$$S = 2[\mathrm{LN}\ (0.52) + \mathrm{LN}\ (0.013) + \mathrm{LN}\ (0.077) + \mathrm{LN}\ (0.375) + \mathrm{LN}\ (0.010)] = 26.28$$

has a X^2 distribution with 2 x 5 = 10 degress of freedom and a joint "P" value of < 0.01 associated with it.

Although, this calculation indicates that the association of prostate cancer and cadmium exposure in these five studies is not likely to be due to chance alone, a definite statement about causation cannot be made considering the limitations of these studies.

Conclusion

Although problems exist in the design and methodology of all 12 epidemiologic studies evaluating the association of cadmium exposure with prostate cancer, six of them are nevertheless consistent in their findings of an elevated risk of prostate cancer. But only in three are the data statistically significant. The remaining six studies are inadequate to evaluate this risk. Because of small sample sizes and a negative dose-response curve in one of the significant studies, the evidence is limited that cadmium is a human carcinogen.

In two studies (Lemen et al. 1976, Holden 1980) where a significant risk of bronchogenic cancer was apparent, there is a suggestion of an association of exposure to cadmium with bronchogenic carcinoma. However, the confounding effects of exposure to arsenic in both these studies makes it questionable that the association is real with respect to renal cancer, and there is sufficient reason to doubt the finding of a significant positive association in the only study that demonstrated such a risk (Kolonel 1976).

QUANTITATIVE ESTIMATION

INTRODUCTION

This quantitative section deals with the unit risk for cadmium in air and the potency of cadmium relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air pollutant is defined as the lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ug/m^3 of the agent in the air that they breathe. This calculation is done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

The data used for the quantitative estimate are taken from one or both of the following: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses with an incidence determined by the extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations including the unit concentration defined above. For practical reasons such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear nonthreshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature probably reflecting the effects of multistage processes on the mutagenic response. The linear nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin). Linearity is also supported when the mode of action of the carcinogen in question is similar to that of the background cancer occurrence in the exposed population.

Because it has the best, albeit limited, scientific basis of any of the current mathematical extrapolation models, a linear nonthreshold model has been adopted as the primary basis for estimating risk at low levels of exposure. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper-limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably by inhalation.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough, but plausible estimate of the upper-limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as an accurate representation of the true cancer risks even when the exposures are accurately defined. The estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

In addition to the multistage model currently used by the CAG for low-dose extrapolation (a detailed description of the procedure is given in Appendix B), three more models, the probit, Weibull, and one-hit, are employed for the purpose of comparison. These models cover almost the entire spectrum of risk estimates that could be generated from the existing mathematical extrapolation models. These models are generally statistical in character and are not derived from the biological arguments, except for the multistage model which has been used to support the somatic mutation hypothesis of carcinogenesis (Armitage and Doll 1954, Whittemore 1978, Whittemore and Keller 1978).

The main differences among these models is the rate at which the response function P(d) approaches zero or P(0) as dose d decreases. For instance, the probit model would usually predict a smaller risk at low-doses than the multistage model because of the difference of the decreasing rate in the low-dose region. However, it should be noted that one could always artificially make the multistage model have the same (or even greater) rate of decrease as the probit model, by making some dose transformation and/or by assuming that some of the parameters in the multistage model are zero. This, of course, is not reasonable without knowing, a priori, what the carcinogenic process for the agent is.

Although the multistage model appears to be the most reasonable or at least the most general model to use, the point estimate generated from this model is of limited value because the issue remains as to what the shape of the dose-response curve is beyond the experimental exposure levels. Furthermore, the point estimates at low doses extrapolated beyond the experimental dose could be extremely unstable and could differ drastically, depending on where the lowest experimental dose is. Since the upper-bound estimates at low doses from the multistage model are relatively more stable than the point estimates, we suggest that the upper-bound estimate of the risk (or the lower-bound estimates of the dose) be used in evaluating the carcinogenic potency of a suspect carcinogen. The upper-bound estimate can be taken as a plausible estimate if the true dose-response curve is actually linear at low doses. The upper-bound estimate means that the risks are not likely to be higher but could be lower if the compound has a concave upward dose-response curve or a threshold at low doses. Another reason one can, at best, obtain an upper-bound estimate of the risk when animal data are used is that the estimated risk is only a conditional probability under the assumption that an animal carcinogen is also a human carcinogen. Therefore, in reality, the actual risk could range from a value near zero to an upper-bound estimate.

PROCEDURES FOR DETERMINING THE CARCINOGENIC POTENCY

Description of the Low-Dose Animal Extrapolation Model

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \ i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficents q_i, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk $P_t(d)$ are calculated by using the computer program GLOBAL79 developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1 \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit q_1 is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satifies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P). In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i \ (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

Selection of Data--

For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed as follows.

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data that has the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \dots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

Calculation of Human Equivalent Dosages from Animal Data--

Following the suggestion of Mantel and Schneiderman (1975), we assume that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the 2/3rds power of the weight as would be the case for a perfect sphere, the exposure in mg/day per 2/3rds power of the weight is also considered to be equivalent exposure. In an animal experiment this equivalent dose is computed in the following manner. Let

L_e = duration of experiment
l_e = duration of exposure
m = average dose per day in mg during administration of the agent (i.e., during l_e), and
W = average weight of the experimental animal

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

Inhalation--When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol, and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

Case 1--Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportional to the breathing rate. In this case the exposure in mg/day maybe expressed as

$$m = I \times v \times r$$

where I = inhalation rate per day in m^3, $v = mg/m^3$ of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations (FASEB 1974) that 25 g mice breathe 34.5 liters/day and 113 g rats breathe 105 liters/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the values of 20 m^3/day is adopted as a standard breathing rate. The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake per kg per day, $i = I/W$, based upon the previous stated relationships, are tabulated as follows:

Species	W	i = I/W
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

Case 2--The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = kW^{2/3}$, where k is a constant independent of species, it follows that

$$m = k\ W^{2/3}vr$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or ug/m^3 in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al. 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice-versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

Calculation of the Unit Risk from Animal Studies--

The 95% upper-limit risk associated with d mg/kg$^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp-(q_1^*d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value we simply find the number of mg/kg$^{2/3}$/day corresponding to one unit of X and substitute this value into the above relationship. Thus, for example, if X is in units of ug/m^3 in the air, we have that for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ mg/kg$^{2/3}$/day, and for case 2, d = 1, when ug/m^3 is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, we may simply use the fact that

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note, an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures and then increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1, 2, \ldots, k$$

and use mg/kg equivalents for the unit risk values.

Model for Estimation of Unit Risk Based on Human Data

If human epidemiologic studies and sufficiently valid exposure information are available for the compound, they are always used in some way. If they show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose. If they show no carcinogenic effect when positive animal evidence is available, then it is assumed that a risk does exist, but it is smaller than could have been observed in the epidemiologic study, and an upper-limit to the cancer incidence is calculated assuming hypothetically that the true incidence is just below the level of detection in the cohort studied, which is determined largely by the cohort size. Whenever possible, human data are used in preference to animal bioassay data.

Very little information exists that can be utilized to extrapolate from high exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), P_0, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in some units, say ppm. The factor B_H is the increased probability of cancer associated with each unit increase of r, the agent in air.

If we make the assumption that R, the relative risk of cancer for exposed workers, compared to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H\,(x_1 + x_2)}{A + B_H\,x_1}$$

or

$$RP_0 = A + B_H\,(x_1 + x_2)$$

where x_1 = lifetime average daily exposure to the agent for the general population, x_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible exposure.

Substituting $P_0 = A + B_H\,x_1$ and rearranging gives

$$B_H = P_0\,(R - 1)/x_2$$

To use this model, estimates of R and x_2 must be obtained from the epidemiologic studies. The value P_0 is derived from the age-cause-specific death rates for combined males found in 1973 U.S. Vital Statistics tables using the life table methodology.

CADMIUM RISK ESTIMATES

Unit Risk Estimate Based on an Animal Study

The bioassay by Takenaka et al. (1982) using male Wistar rats and cadmium chloride aerosol was chosen for estimating the quantitative unit risk of cadmium. This was the only positive animal inhalation study with cadmium and/or cadmium compounds, with a dose-response trend of primary lung carcinomas to animals continuously exposed to $CdCl_2$ aerosols for 18 months. The primary lung carcinomas were histologically differentiated as adenocarcinomas, epidermoid carcinomas, combined epidermoid and adenocarcinomas, and mucoepidermoid carcinomas, but were combined for this unit risk analysis. The incidences of total primary lung carcinomas was 15% (6/39), 53% (20/38), and 71% (25/35) for the low (12.5 ug/m^3), medium (25 ug/m^3), and high (50 ug/m^3) exposure groups, respectively. No tumors were found among 38 controls.

In calculating an upper-limit unit risk estimate for humans, dose is calculated on a lifetime continuous basis with 2 years considered a full lifetime exposure for rats. Thus, by multiplying by 0.75, the measured concentrations of 13.4 ug/m^3, 25.7 ug/m^3, and 50.8 ug/m^3 for the three dose groups, the lifetime continuous exposure can be estimated as 10.05 ug/m^3, 19.3 ug/m^3, and 38.1 ug/m^3, respectively. The corrections for animal to human weight differences are given below.

In transforming from animal exposure to human equivalence, the method for treating inhalation of an aerosol [presented earlier in the section for calculation of human equivalent dosages from animal data (Case 1)], assumes aerosols to be absorbed proportional to the breathing rate. This breathing rate is also given there for 113 g rats as 0.105 m^3/day. For the Wistar rats used in the Takenaka et al. bioassay the average weights at 18 months were 424.6 g (for the 13.4 ug/m^3 group), 437.6 g (for the 25.7 ug/m^3 group), and 424.3 g (for the 50.8 ug/m^3 group). Adjusting for these weights we use the formula

$$I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

where I = the daily inhalation rate of a rat weighing W kilograms. For the three groups the I values are 0.254 m^3/day, 0.259 m^3/day, and 0.254 m^3/day, respectively. Combining these with the lifetime continuous exposure estimates above, we estimate daily exposure as 2.55 ug/day, 5.00 ug/day, and 9.68 ug/day, respectively. Equivalently, we can estimate dose on a ug/kg/day basis as 6.0 ug/kg/day, 11.4 ug/kg/day, and 22.8 ug/kg/day.

Based on the above data, the 95% upper-limit unit risk for induced cancer based on cadmium chloride exposure is $q_1^* = 6.3 \times 10^{-2}(\text{ug/kg/day})^{-1}$ based on animals using the linearized multistage model. When transformed to equivalent human dose, the CAG method requires multiplying q_1^* by the weight ratio factor $(W_h/W_a)^{1/3}$, where W_h = weight of a human, which we assume to be 70 kg. Thus,

$$q_h^* = q_1^* \ (W_h/W_a)^{1/3} = 6.3\times10^{-2}(70/0.429)^{1/3} = 3.4\times10^{-1}(\text{ug/kg/day})^{-1}$$

Thus, using the linearized multistage model, the 95% upper-limit unit risk estimate for induced cancers based on cadmium chloride exposure is q_h^* = 3.4×10^{-1}. If we believe that the Cd^{++} ion is the carcinogenic agent and not the $CdCl_2$ molecule, then we must adjust for the weight of the two chloride ions. In this case the molecular weight contribution of cadmium to the total molecular weight is 112.4/183.3 = 0.613. The interpretation in terms of risk is that a q_h* for the cadmium ion based on inhalation of $CdCl_2$ is

$$q_h^* = 3.4 \times 10^{-1}(\text{ug/kg/day})^{-1}/0.613 = 5.5 \times 10^{-1}(\text{ug/kg/day})^{-1}$$

This can be converted back to human exposure in terms of ug/m^3 by assuming that a 70 kg human breathes 20 m^3 air/day. Thus,

$$q_h^* = 3.4 \times 10^{-1}(ug/kg/day)^{-1} \times \frac{1}{70\ kg} \times \frac{20\ m^3}{day} =$$

$$9.7 \times 10^{-2}(ug/m^3)^{-1}$$

for cadmium chloride exposure, and

$$q_h{}^* = 9.7 \times 10^{-2}(ug/m^3)^{-1}/0.613 = 1.6 \times 10^{-1}(ug/m^3)^{-1}$$

based on inhalation exposure to the cadmium ion. Therefore, the unit risk from the inhalation of one microgram of elemental cadmium per cubic meter of air is approximately

$$R = 1 - \exp -(0.16 \times 1) = 0.15$$

This is an upper-bound estimate of risk based upon the best direct experimental evidence presently available. Using other dose-response models to estimate risk (as shown in Appendix A) can give considerably lower estimates than obtained using the upper-bound multistage model. However, there is no direct evidence suggesting that these alternative models provide a more rational basis for estimating risk than the upper-bound multistage model. It must be kept in mind that the alternative models have the potential for seriously underestimating the true risk at low levels of environmental exposure to cadmium.

Unit Risk Estimate Based on a Human Study

Data Base--

At the present time the strongest evidence in humans suggesting a cadmium-induced carcinogenic response is found in the Lemen et al. (1976) study. It was observed in a cohort of cadmium smelter workers with more than 2 years of exposure that 12 deaths were due to a malignant neoplasm of the respiratory system, while only 5.11 would have been expected based upon the age, calendar-time, and cause-specific mortality rates for the total United States white male population (relative risk = 2.34). Assuming that the U.S. white male population is a valid control population for the cohort of cadmium smelter workers, we find that the probability of obtaining 12 or more respiratory cancer deaths is P = 0.0063 using the exact Poisson Test.

A number of problems exist in using these data to obtain a quantitative estimate of human respiratory cancer risk due to cadmium exposure. Among them are the following:

1. The smoking histories of the cadmium workers are unknown, and if their smoking rates were substantially higher then white U.S. male averages, this fact alone could explain the twofold increase in relative risk.
2. The exposure to cadmium is confounded with exposure to arsenic, a known respiratory carcinogen.
3. Very limited evidence exists concerning the exposure rate and the duration of exposure for the members of the cohort.
4. No exposure estimates exist for individuals.
5. To obtain an estimate of risk, a mathematical model must be assumed that cannot be evaluated for goodness of fit in any reasonable manner using the available data.

In spite of these considerable shortcomings, it was still felt that a risk estimate based on the limited and potentially biased data base could be of use for the following reasons:

1. The observed human respiratory cancer response corresponded to the animal response in regard to site, which increases the likelihood that the response is real.
2. All the factors that are potentially biasing would work to increase the apparent cadmium-induced cancer risk. Thus, such a risk estimate should be considered an upper-bound estimate. If this upper-bound estimate is lower than the one obtained in the animal experiment, it should be used in preference to the animal estimate.

Another approach for obtaining a risk estimate would be to use the information on prostate cancer. In the cohort studied by Lemen et al. (1976), after a lapse of 20 years from initial exposures, 4 cases were observed versus 0.88 expected (SMR = 4.55), which is a statistically significant finding ($P < 0.01$). Prostate cancer was also marginally significant in the Holden (1980) study, but exposure information for individuals in his cohort was not well-enough defined to permit a risk estimate. Use of the prostate cancer data avoids the difficulties in numbers 1 and 2 relating to respiratory system cancers, but the other difficulties remain.

Risk Model--

We make the following assumption to obtain a simple risk model. It is assumed that an average daily lifetime exposure increases the relative risk over the entire lifespan by an amount that is linearly related to the exposure. This model would tend to overestimate a risk unless early exposures in one's lifetime are much more critical than exposure during the working lifetime. Under this model, the unit risk estimate has the following simple form:

$$P = \frac{P_0 (R-1)}{X}$$

where P_0 is the U.S. lifetime respiratory or prostate cancer risk in the absence of cadmium exposure, R is the relative risk for cadmium smelter workers, and X is the lifetime average exposure received by the cadmium workers.

For respiratory cancer the value for P_0 is 0.036 based upon 1973 U.S. Vital Statistics data. The term R, the relative risk, is 2.34 with a 95% confidence interval of 1.35 to 4.10 based upon the exact Poisson distribution.

For prostate cancer the value of P_0 is 0.0187 using the same data source and the relative risk is 4/0.88 = 4.55.

To estimate the average lifetime exposure on a continuous basis, a number of factors need to be estimated. They are:

The average age, t, of the cohort at the end of the observation period.

The average duration, d, of exposure for the cohort in years.

The average exposure rate, e, on the job.

The fraction, f, of time per year exposed on the job.

Given these factors, an estimate of the average exposure rate over the cohort's lifetime is

$$X = def/t$$

Unfortunately, information is very limited concerning any of the above factors. The approach to be taken is to use the limited information to make an educated guess as to what is the best estimate of each quantity. In addition, an upper- and lower-bound is given for each quantity, which most likely brackets the true value. We shall discuss each of the following terms in order.

Average age, t, at the end of the observation period--The only hints on the age of the cohort is that the people were employed for at least 2 years during the period 1940 to 1970 and were followed up to the start of 1974.

As an upper-bound it is assumed that the average age at the end of the observation period is 70. The lower-bound is set at 40 and the best guess is taken be the midpoint, or 55.

Average duration, d, of exposure--We know that each individual was exposed for at least 2 years and that the four individuals that died of prostatic cancer were exposed for 4, 13, 17, and 18 years. If we make the potentially biased assumption that these four observations are a random sample from the total smelter worker population and obtain a 95% confidence interval around the mean of the sample, we have that best estimate is 13 with bounds from 3 to 23.

Average exposure rate, e, on the job--Information concerning exposure rates during the appropriate tie frame that we have at our disposal is taken from the Lemen et al. (1976) study.

> In 1947, a previously cited study by Princi reported an average air concentration of cadmium fumes that ranged from 0.04 to 6.59 mg/m^3 and of cadmium dust at 17.23 mg/m^3, with one man exposed 2-3 hr/day to an average cadmium dust concentration of 31.3 mg/m^3. Most operations, however, had concentrations lower than 1.5 mg/m^3.

As a low estimate we take the geometric mean of the range of the cadmium fumes, $(0.04 \times 6.59)^{1/2} = 0.5$ mg/m^3. As a best estimate we use the 1.5 mg/m^3 and as a upper-bound, 6 mg/m^3.

Fraction, f, of time per year exposed on the job--We will assume that as a low, "best," and high estimate the number of hours per day exposed on the job is 2, 4, and 8 hours, respectively, and the number of days exposed per year as 120, 180, and 240 days, respectively.

Estimation of Unit Risk--

The values assumed for each parameter used in the estimation of the unit risk based on respiratory cancer data is shown in Table 16. For prostate cancer the "best" estimate of risk can be obtained, using the equation

TABLE 16. "BEST" ESTIMATE AND BOUNDS ON TERMS USED TO ESTIMATE UNIT RISK FROM RESPIRATORY CANCER DATA IN THE LEMEN et al. STUDY

Factor	Value Maximizing Risk	Value Giving "Best" Estimate of Risk	Value Minimizing Risk
t = age at end of observation period	70	55	40
d = years duration exposure	3	13	23
e = exposure rate on job (mg/m^3)	0.5	1.5	6
h = hours in exposure per day	2	4	8
n = days worked per year	120	180	240
f = hn÷(24 x 365) fraction of time per years exposed	0.0274	0.0822	0.2192
x = 1,000 def/t average lifetime exposure x ug/m^3	0.587	29.1	756
R = relative risk	4.10	2.34	1.35
		Unit Risk Based on Above Values	
P = 0.036(R-1)/I =	1.9×10^{-1}	1.6×10^{-3}	1.7×10^{-5}

given previously for estimating the unit risk, by multiplying the risk for respiratory cancer by the factor

$$(R_p - 1)P_{o,p}/(R_r - 1)P_{o,r} = (4.55 - 1)0.0187/(2.34 - 1)0.036$$

where the subscripts p and r refer to prostate and respiratory cancer data, respectively. Therefore, the "best estimate of the unit risk based on prostate cancer data is $1.6 \times 10^{-3} \times 1.38 = 2.2 \times 10^{-3}$. The effect of compounding, especially multiplying together, a series of assumptions that consistently overestimates or underestimates the true values of parameters used to estimate risk leads to very different estimates. This is true even though the mathematical model itself, a major source of uncertainty, remains the same. However, it is highly unlikely that either extreme is close to the true value. We take as our estimate the value obtained by compounding the series of best guesses. Even though a single term may not be conservative, the overall result is probably more reasonable than either of the extremes.

One final correction is needed. We assume that human exposure was to cadmium oxide, CdO; thus, the risk to elemental cadmium is increased by the ratio

$$(CdO/Cd) = (128.4/112.4) = 1.14$$

with a corresponding unit risk estimate of

$$P = 1.65 \times 10^{-3} \times 1.14 = 1.88 \times 10^{-3} \text{ from respiratory data}$$

and

$$P = 2.2 \times 20^{-3} \times 1.14 = 2.51 \times 10^{-3} \text{ from prostate data.}$$

This estimate is two orders of magnitude lower than the estimate based on the rat inhalation sutdy, which was 0.156. Some of this difference might be explained as a generalized difference in the biological activity between

$CdCl_2$ in rats and Cd dust and fumes in humans. In any event, our final estimate is based on the human study, which is used for the relative potency calculation.

RELATIVE POTENCY

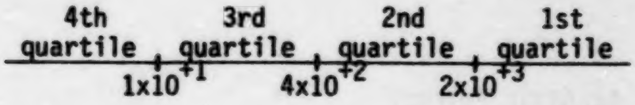
One of the uses of unit risk is to compare the potency of carcinogens. In this section the potency of cadmium is compared with that of other chemicals that the CAG has evaluated as suspect carcinogens. To estimate the relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight and the resulting number expressed in terms of $(\text{mMol/kg/day})^{-1}$. This is called the relative potency index.

Figure 2 is a histogram representing the frequency distribution of potency indicies of 53 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 17. When human data were available for a compound, they have been used to calculate the index. When no human data are available, animal oral studies and animal inhalation studies have been used in that order. Animal oral studies are selected over animal inhalation studies because most of the chemicals have animal oral studies; this furnishes a more consistent basis for potency comparisons.

The potency index for cadmium based on the Lemen et al. (1976) study of cadmium smelter workers is $7.4 \times 10^{+2}$ $(\text{mMol/kg/day})^{-1}$. This is derived as follows: the slope estimate from the human study, 1.88×10^{-3} $(\text{ug/m}^3)^{-1}$, is first converted to units of $(\text{mg/kg/day})^{-1}$, assuming a breathing rate of 20 m^3 of air per day and a 70 kg person.

$$1.88 \times 10^{-3}(\text{ug/m}^3)^{-1} \times \frac{1 \text{ day}}{20 \text{ m}^3} \times \frac{1 \text{ ug}}{10^{-3} \text{ mg}} \times 70 \text{ kg}$$

$$= 6.65(\text{mg/kg/day})^{-1}$$


4th quartile
3rd quartile
2nd quartile
1st quartile
1x10^+1
4x10^+2
2x10^+3

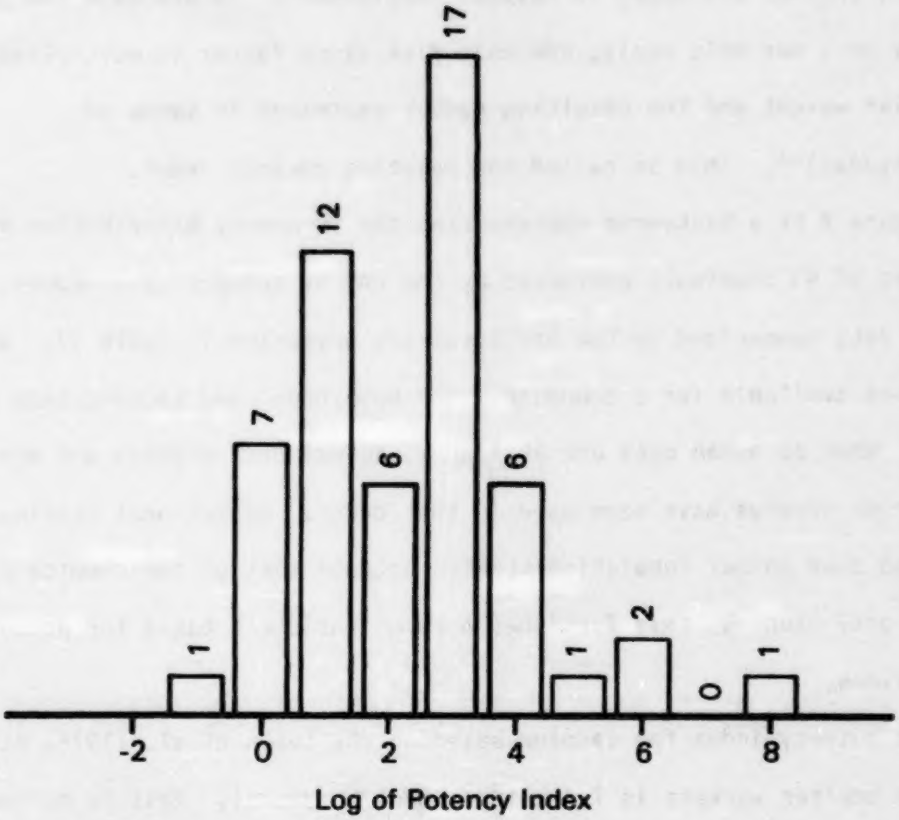


Figure 2. Histogram representing the frequency distribution of the potency indices of 53 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 17. RELATIVE CARCINOGENIC POTENCIES AMONG 53 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS[1,2,3]

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log$_{10}$ Index)
Acrylonitrile	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9x10^{+5}$	+6
Aldrin	11.4	369.4	$4x10^{+3}$	+4
Allyl Chloride	$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	11.5	252.3	$3x10^{+3}$	+3
Benzene	$5.2x10^{-2}$(W)	78	$4x10^{0}$	+1
Benzidine	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	4.86	9	$4x10^{+1}$	+2
Cadmium	6.65(W)	112.4	$7x10^{+2}$	+3
Carbon Tetrachloride	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	1.61	409.8	$7x10^{+2}$	+3
Chlorinated Ethanes				
1,2-dichloroethane	$6.90x10^{-2}$	98.9	$7x10^{0}$	+1
hexachloroethane	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,2,2-tetrachloroethane	0.20	167.9	$3x10^{+1}$	+1
1,1,1-trichloroethane	$1.6x10^{-3}$	133.4	$2x10^{-1}$	-1
1,1,2-trichloroethane	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
Chloroform	$7x10^{-2}$	119.4	$8x10^{0}$	+1
Chromium	41	104	$4x10^{+3}$	+4
DDT	8.42	354.5	$3x10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4x10^{+2}$	+3
1,1-dichloroethylene	$1.47x10^{-1}$(I)	97	$1x10^{+1}$	+1
Dieldrin	30.4	380.9	$1x10^{+4}$	+4

(continued on the following page)

TABLE 17. (continued)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ Index)
Dinitrotoluene	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	9300(I)	115	$1x10^{+6}$	+6
Ethylene Dibromide (EDB)	8.51	187.9	$2x10^{+3}$	+3
Ethylene Oxide	0.63(I)	44.0	$3x10^{+1}$	+1
Formaldehyde	$2.14x10^{-2}$(I)	30	$6x10^{-1}$	0
Heptachlor	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane				
technical grade	4.75	290.9	$1x10^{+3}$	+3
alpha isomer	11.12	290.9	$3x10^{+3}$	+3
beta isomer	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	1.33	290.9	$4x10^{+2}$	+3
Nickel	1.15(W)	58.7	$7x10^{+1}$	+2
Nitrosamines				
Dimethylnitrosamine	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	5.43	158.2	$9x10^{+2}$	+3
N-nitrosopyrrolidine	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	32.9	117.1	$4x10^{+3}$	+4
N-nitroso-N-methylurea	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	4.34	324	$1x10^{+3}$	+3

(continued on the following page)

TABLE 17. (continued)

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ Index)
Phenols				
2,4,6-trichlorophenol	1.99×10^{-2}	197.4	4×10^{0}	+1
Tetrachlorodioxin	4.25×10^{5}	322	$1 \times 10^{+8}$	+8
Tetrachloroethylene	5.31×10^{-2}	165.8	9×10^{0}	+1
Toxaphene	1.13	414	$5 \times 10^{+2}$	+3
Trichloroethylene	1.26×10^{-2}	131.4	2×10^{0}	0
Vinyl Chloride	1.75×10^{-2}(I)	62.5	1×10^{0}	0

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

2. The potency index is a rounded-off slope in (mMol/kg/day)$^{-1}$ and is calculated by multiplying the slopes in (mg/kg/day)$^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

Multiplying by the molecular weight of 112.4 gives a potency index of $7.4 \times 10^{+2}$. Rounding off to the nearest order of magnitude gives a value of 10^{+3}, which is the scale presented on the horizontal axis of Figure 2. The index of $7.4 \times 10^{+2}$ lies in the second quartile of the 53 suspect carcinogens.

Ranking of the relative potency indicies is subject to the uncertainty of comparing estimates of potency of different chemicals based on different routes of exposure to different species using studies of different quality. Furthermore, all the indicies are based on estimates of low-dose risk using the linearized multistage extrapolation model fitted to the data at relatively high doses. Thus, relative potencies could be different at high exposures, where non-linearities in the dose-response curves could exist.

APPENDIX A

COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimate of unit risk from animals presented in the body of this document is calculated by the use of the linearized multistage model. The reasons for its use have been detailed therein. Essentially, it is part of a methodology that estimates a conservative linear slope at low extrapolation doses and is consistent with the data at all dose levels of the experiment. It is a nonthreshold model holding that the upper-limit of risk predicted by a linear extrapolation to low levels of the dose-response relationship is the most plausible upper-limit for the risk.

Other models have also been used for risk extrapolation. Three nonthreshold models are presented here: the one-hit, the log-Probit, and the Weibull. The one-hit model is characterized by a continuous downward curvature but is linear at low doses. It can be considered the linear form or first stage of the multistage model because of its functional form. Because of this and its downward curvature, it will always yield estimates of low level risk which are at least as large as those of the multistage model. Further, whenever the data can be fit adequately by the one-hit model, estimates from the two procedures will be comparable.

The other two models presented below are the log-Probit and the Weibull. They are often used to fit toxicological data in the observable range, because of the general "S" curvature. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-Probit model was originally used in problems of biological assay such as the assessment of potency of toxicants and drugs and has usually been used to estimate such values as percentile lethal dose or percentile effective dose. Its development was strictly empirical, i.e., it was observed that several log dose-response relationships followed the cumulative normal probability distribution function, Φ . In fitting the cancer bioassay data, assuming on independent background, this becomes

$$P(D;a,b,c) = c + (1-c)\ \Phi\ (a+b\log_{10} D) \quad a,b > 0 \le c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standarized mean of individual tolerances, and b is an estimate of the log dose-Probit response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single normal cell into a cancer cell. It has the probability distribution function

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates. The estimate a represents the background or zero dose rate, and the parameter estimated by b represents the linear component or slope of the dose-response model. In discussing the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1-\exp-(bd^{k}) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, this model yields low-dose estimates of risks usually significantly lower than either the multistage or one-hit models, which are linear at low doses. All three of these models usually project risk estimates significantly higher at low exposure levels than those from the log-Probit.

The estimates of added risk for low doses for these models are given in Table 18 for the cadmium chloride rat inhalation studies by Takenaka et al. Both maximum likelihood estimates and 95% upper confidence limits are presented. The results show that the maximum likelihood estimates of risk for the log-Probit model are all less than those for the other models and this difference increases sharply at low doses. The one-hit model yields maximum likelihood estimates slightly higher than from the multistage model, with the Weibull somewhat lower.

TABLE 18. ESTIMATES OF LOW-DOSE RISK TO HUMANS EXPOSED TO CADMIUM CHLORIDE BASED ON MALE WISTAR RATS FROM THE TAKENAKA ET AL. INHALATION STUDY DERIVED FROM FOUR DIFFERENT MODELS

	Maximum Likelihood Estimates of Additional Risks				95% Upper Confidence Limit of Additional Risks			
Dose ug/m^3	Multistage Model	One-Hit Model	Weibull Model	Log-Probit Model	Multistage Model*	One-Hit Model	Weibull Model	Log-Probit Model
10^{-4}	$5.5x10^{-6}$	$8.1x10^{-6}$	$1.9x10^{-7}$	0	$9.7x10^{-6}$	$1.0x10^{-5}$	$1.3x10^{-6}$	$1.2x10^{-38}$
10^{-3}	$5.5x10^{-5}$	$8.1x10^{-5}$	$4.1x10^{-6}$	0	$9.7x10^{-5}$	$1.0x10^{-4}$	$2.6x10^{-5}$	$8.9x10^{-25}$
10^{-2}	$5.5x10^{-4}$	$8.1x10^{-4}$	$8.8x10^{-5}$	$2.0x10^{-15}$	$9.7x10^{-4}$	$1.0x10^{-3}$	$3.8x10^{-4}$	$4.4x10^{-14}$
10^{-1}	$5.5x10^{-3}$	$8.1x10^{-3}$	$1.9x10^{-3}$	$1.3x10^{-7}$	$9.7x10^{-3}$	$1.0x10^{-2}$	$5.9x10^{-3}$	$1.5x10^{-6}$
1	$5.5x10^{-2}$	$7.8x10^{-2}$	$3.9x10^{-2}$	$7.0x10^{-3}$	$9.2x10^{-2}$	$9.5x10^{-2}$	$8.1x10^{-2}$	$2.3x10^{-2}$

* $q_h^* = 9.7 \times 10^{-2}(ug/m^3)^{-1}$ for the multistage model; $P(d) = 1 - e^{-q_h^* d}$

APPENDIX B

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) CLASSIFICATION FOR WEIGHT-OF-EVIDENCE FOR CARCINOGENICITY OF A SUSPECTED CARCINOGEN

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN HUMANS

The degrees of evidence for carcinogenicity from studies in humans are categorized as:

1. Sufficient evidence of carcinogenicity, which indicates that there is a causal relationship between the agent and human cancer.
2. Limited evidence of carcinogenicity, which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded.
3. Inadequate evidence, which indicates that one of three conditions prevailed: (a) there were few pertinent data; (b) the available studies, while showing evidence of association, did not exclude chance, bias, or confounding; (c) studies were available which do not show evidence of carcinogenicity.

ASSESSMENT OF EVIDENCE OF CARCINOGENICITY FROM STUDIES IN EXPERIMENTAL ANIMALS

These assessments are classified into four groups:

1. Sufficient evidence of carcinogenicity, which indicates that there is an increased incidence of malignant tumors: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels; or (c) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.

2. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) the studies involve a single species, strain, or experiment; or (b) the experiments are restricted by inadequate dosage levels, inadequate duration to exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting, or (c) the neoplasms produced often occur spontaneously and, in the past, have been difficult to classify as malignant by histological criteria alone (e.g., lung and liver tumors in mice).

3. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect; or that within the limits of the tests used, the chemical is not carcinogenic. The number of negative studies is small, since, in general, studies that show no effect are less likely to be published than those suggesting carcinogenicity.

4. No data indicates that data were not available to the working group.

EVALUATION OF CARCINOGENIC RISK TO HUMANS

At present, no objective criteria exist to interpret data from studies in experimental animals or from short-term tests directly in terms of human risk. Thus, in the absence of sufficient evidence from human studies, evaluation of the carcinogenic risk to humans was based on consideration of both the epidemiological and experimental evidence. The breadth of the categories of evidence defined above allows substantial variation within each. The decisions reached by the group regarding overall risk incorporated these differences, even though they could not always be reflected adequately in the placement of an exposure into a particular category.

The chemicals, groups of chemicals, industrial processes, or occupational exposures were thus put into one of three groups:

Group 1

The chemical, group of chemicals, industrial process, or occupational exposure is carcinogenic to humans. This category was used only when there was sufficient evidence from epidemiological studies to support a causal association between the exposure and cancer.

Group 2

The chemical, group of chemicals, industrial process, or occupational exposure is probably carcinogenic to humans. This category includes exposures for which, at one extreme, the evidence of human carcinogenicity is almost "sufficent," as well as exposures for which, at the other extreme, it is inadequate. To reflect this range, the category was divided into higher (Group A) and lower (Group B) degrees of evidence. Usually, category 2A was reserved for exposures for which there was at least limited evidence of carcinogenicity to humans. The data from studies in experimental animals played an important role in assigning studies to category 2, and particularly those in Group B; thus, the combination of sufficient evidence in animals and inadequate data in humans usually resulted in a classification of 2B.

In some cases, the working group considered that the known chemical properties of a compound and the results from short-term tests allowed its transfer from Group 3 to 2B or from 2B to 2A.

Group 3

The chemical, group of chemicals, industrial process, or occupational exposure cannot be classified as to its carcinogenicity to humans.

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